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INVESTIGATION OF THE 17-OXOSTEROIDS

IN THE HEPATIC PORPHYRIAS

by

JAMES W. PAXTON, B.Sc.

Thesis Submitted for the Degree of

Doctor of Philosophy

of the

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SUMMARY OF THESIS

During the past eighty years, there has been much clinical and experimental evidence presented by a wide variety of investigations confirming the deleterious interplay of endocrine and genetic factors in the natural history of these diseases known as the porphyrias. The porphyrin-inducing 5β -H oxosteroids that have been identified delineate one category of endogenous agents through which such an endocrine-genetic interplay may be affected in some subjects, and it is the purpose of this investigation to examine the relevance of the part played, if any, by some of these 5α -H and 5β -H 17-oxosteroids in the pathogenesis of the porphyrias in humans.

Preliminary experiments included the setting-up and evaluation of methods for the measurement of four 17-oxosteroid sulphates and eight 17-oxosteroid glucuronides in urine and the measurement of their levels in urine in seventy-five normal subjects, consisting of thirty-eight males and thirty-seven females. A pattern similar to that obtained by Hamburger (1948) for the total urinary 17-oxosteroids excretion of different age groups was obtained for the individual urinary 17-oxosteroid levels in this control group. These methods were then applied to the study of the urinary levels of these 17-oxosteroids in patients suffering from acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and porphyria cutanea tarda (PCT). In total, thirty-four patients with AIP, five patients with HCP, two

patients with VP and fourteen patients with PCT were investigated. Of the thirty-four patients with AIP, a total of twenty-three had elevated levels of certain of these 17-oxosteroids, especially dehydroepiandrosterone sulphate (DHAS) and aetiocholanolone glucuronide (EG) and sulphate (ES) in the urine. The seven patients with AIP who were investigated during or shortly after attack had levels of urinary 17-oxosteroids well beyond the range of the normal control group. Similar results were obtained with the five patients with HCP, with the exception that the elevations of these 17-oxosteroids were observed only in the urine of the three patients who were in attack and not in those who were in remission. In one patient with AIP and two patients with HCP from whom serial 24-hour urine specimens were obtainable, significant linear correlations were found between the values of the ratio of urinary aetiocholanolone to androsterone and the levels of 6-aminolaevulic acid (ALA) and porphobilinogen (PBG) in the individual urines. The two patients with VP who were investigated were in remission and did not show any elevations of these steroids in the urine. The fourteen patients with PCT who were studied did not show any elevations of the urinary levels of these 17-oxosteroids, but it was noted that this group tended to excrete lower levels of steroids than the similarly aged normal controls.

More specific investigations were undertaken with one male patient who had suffered from several acute attacks of porphyria. During those attacks, his DHAS and EG were the predominantly raised urinary steroids. After treatment with dexamethasone,

which suppresses the activity of the adrenal cortex, his clinical condition improved, his urinary 17-oxosteroid levels fell sharply, corresponding with an equally sharp fall in urinary porphyrins and precursors (ALA and PBG).

Having established these irregularities in the levels of 17-oxosteroids in the urine of some of these porphyric patients, this pathway of research was further pursued by setting up and carefully evaluating a method for estimating the four main 17-oxosteroid sulphates in plasma. As with the urinary studies, a similar control group of wide age range consisting of fifty-three males and thirty-nine females was studied initially. Some of the subjects from the control group were investigated for fluctuations due to diurnal variation and for cyclic fluctuations due to different phases of the normal menstrual cycle. Having established normal values for the concentrations of these 17-oxosteroid sulphates in plasma, investigation of their concentrations in the plasma of patients suffering from hepatic porphyria was undertaken. As with the urinary results, no elevations of the levels of these steroids beyond the normal range were observed in the plasma of patients with PCT but, in the plasma of patients with AIP, elevations of these steroids, predominantly DHAS and ES, were observed, in most cases corresponding with similar urinary elevations.

These results, along with the observations of other investigations, do suggest that although the primary genetic defect may not be an endocrine one, the effects of many of the factors involved in the initiation, provocation and aggravation of the hepatic porphyrias may be mediated through interaction with an endogenous regulatory

system of steroid metabolism in the liver. This interference with normal endogenous steroid metabolism may result in direct overproduction and accumulation in the liver of a steroid with strong porphyrinogenic inducing activity (perhaps aetiocholanolone or dehydroepiandrosterone) or indirectly in antagonism of the hypophysis, hypothalamus and endocrine glands which form a functional unit for the homeostatic regulation of the plasma levels of hormones.

The suggestion is also put forward that dehydroepiandrosterone or a close metabolite may possibly act as a constant stimulus to haem biosynthesis in the human hepatic cell.

ABBREVIATIONS

A	Androsterone
ACTH	Adrenocorticotrophic hormone
ADH	Antidiuretic hormone
AG	Androsterone glucuronide
AIA	2-Allyl-2-isopropyl acetamide
AIP	Acute intermittent porphyria
ALA	δ -Aminolaevulic acid
ALA.D	ALA. Dehydrase (Activity)
ALA.S	ALA. Synthetase (Activity)
AS	Androsterone sulphate
ATP	Adenosine triphosphate
BSP	Sulphobromophthalein
Copro	Coproporphyrin
C.V.	Coefficient of variation
DDC	3,5-Diethoxycarbonyl-1,4-dihydrocollidine
DHA	Dehydroepiandrosterone
DHAG	DHA glucuronide
DHAS	DHA sulphate
DNA	Deoxyribonucleic acid
d.p.m.	Disintegrations per minute
E	Aetiocholanolone
EG	Aetiocholanolone glucuronide
ES	Aetiocholanolone sulphate
EPI	Epiandrosterone
EPIG	Epiandrosterone glucuronide
EPIS	Epiandrosterone sulphate

g.l.c.	Gas-liquid chromatography
HCP	Hereditary coproporphyria
HS CoA	Coenzyme A
m RNA	Messenger ribonucleic acid
NADP ⁺	Nicotinamide-adenine dinucleotide phosphate
NADPH	Reduced NADP ⁺
NGS	Neopentyl glycol succinate (liquid phase)
11-DA	11-Oxoandrosterone
11-OAG	11-DA glucuronide
11-OE	11-Oxoetiocholanolone
11-OEG	11-OE glucuronide
11-OHA	11-Hydroxyandrosterone
11-OHAG	11-OHA glucuronide
11-OHE	11-Hydroxyetiocholanolone
11-OHEG	11-OHE glucuronide
17-OS	17-Oxosteroid
p	Probability of an event being due to chance alone
PBG	Porphobilinogen
PCT	Porphyria cutanea tarda
PREG	Pregnenolone
PREG S	Pregnenolone sulphate
PROTO	Protoporphyrin
QF-1	Methyl fluoralkyl silicone polymer (liquid phase)
r	Correlation coefficient
RNA	Ribonucleic acid
S.D.	Standard deviation
TMCS	Trimethylchlorosilane
TMSE	Trimethyl silyl ether

UDP	Uridine diphosphate
UDPGA	Uridine diphosphoglucuronic acid
Uro	Uroporphyrin
VP	Variegate porphyria

SYSTEMATIC NAMES FOR STEROIDS

<u>Trivial Name</u>	<u>Systematic Name</u>
Aetiocholandiols:	5 β -Androstane-3 α ,17 β -diol
Aetiocholanolone:	3 α -Hydroxy-5 β -androstan-17-one
Δ^4 -Androstenediol:	Androst-4-ene-3 β ,17 β -diol
Androstenedione:	Androst-4-ene-3,17-dione
Androsterone (A):	3 α -Hydroxy-5 α -androstan-17-one
Cholesterol:	Cholest-5-en-3 β -ol
Cortisone:	17 α ,21-Dihydroxypregn-4-ene-3,11,20-trione
Dehydroepiandrosterone (DHA):	3 β -Hydroxyandrost-5-en-17-one
Deoxycorticosterone:	21-Hydroxypregn-4-ene-3,20-dione
Dexamethasone:	16 α -Methyl-9 α -fluoro-1,4-pregnadiene- -11 β ,17 α ,21-triol-3,20-dione
Epiandrosterone:	3 β -Hydroxy-5 α -androstan-17-one
Hydrocortisone:	11 β ,17 α ,21-Trihydroxypregn-4-ene-3,20-dione
11 β -Hydroxyaetiocholanolone:	3 α ,11 β -Dihydroxy-5 β -androstan-17-one
11 β -Hydroxyandrostenedione:	11 β -Hydroxyandrost-4-ene-3,17-dione
11 β -Hydroxyandrosterone:	3 α ,11 β -Dihydroxy-5 α -androstan-17-one
Oestradiol:	Oestra-1,3,5(10)-triene-3,17 β -diol
Oestriol:	Oestra-1,3,5(10)-triene-3,16 α ,17 β -triol
Oestrone:	3-Hydroxyoestra-1,3,5(10)-trien-17-one
11-Oxoetiocholanolone:	3 α -Hydroxy-5 β -androstane-11,17-dione
11-Oxoandrosterone:	3 α -Hydroxy-5 α -androstane-11,17-dione
Pregnanediol:	5 β -Pregnane-3 α ,20 α -diol

Trivial NameSystematic Name

Pregnanetriol:	5 β -Pregnane-3 α ,17 α ,20 α -triol
Pregnanolone:	3 α -Hydroxy-5 β -pregnan-20-one
Pregnenolone:	3 β -Hydroxypreg-5-en-20-one
Progesterone:	Pregn-4-ene-3,20-dione
Testosterone:	17 β -Hydroxyandrost-4-en-3-one

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SECTION I

INTRODUCTION

A. HISTORICAL BACKGROUND.

The porphyrias are a group of diseases, mainly hereditary in origin, which have many different symptoms. In some of them the only problem is an undue sensitivity of skin to sunlight; in others the normal life of a patient may be shattered by devastating attacks of abdominal pain, paralysis of limbs and profound mental upset. Biochemically these diseases are characterized by excessive excretion of porphyrins and/or precursors in the urine or the faeces.

The first actual reference to porphyrin in history was made by Scherer in 1841, when he added concentrated sulphuric acid to dried blood and washed the precipitate free of iron. The iron-free residue, after it was treated with alcohol, took on a blood-red colour; thus, he was able to show that the red colour of blood was not caused by iron. Three years later, this purple-red fluid was named iron-free haematin by Mulder (1844). The spectrum of this substance and its remarkable fluorescence were described by Thudichum in 1867. The word 'porphyrin' (derived from the Greek 'porphyros', meaning reddish-purple) was first used by Hoppe-Seyler in 1871 to describe the purple constituent of iron-free haematin. He named this substance haematoporphyrin. The first association of this pigment in urine with a disease in man was recorded by Baumstark (1874), who thoroughly investigated the urine of a case presented by Schultz in 1874. Schultz described this patient as a thirty-three year old weaver who had suffered from skin photosensitivity since the age of three months. His

spleen was enlarged and he passed a wine-red urine. This case was described by Schultz under the name 'Pemphigus leprosus' but, from the description given, it seems clear that it was a case of congenital porphyria. This diagnostic interpretation is also made certain by the autopsy record of intense red-brown discolouration of the skeleton.

Sulphonal was introduced in 1888 into medical therapy as a hypnotic and tranquilliser. In 1889, Stokvis described the case history of an elderly woman who passed a dark red urine after taking sulphonal. The patient became paralysed and subsequently died. He considered that the pigment in her urine was similar to haematoporphyrin. In the following year, Harley (1890) described a fatal case of an unusual form of nervous disturbance associated with a dark red urine in a young woman, aged 27, at the Edinburgh Royal Infirmary. This woman had also been given sulphonal, and she showed many of the neurological features of porphyria. In the following years, an increasing number of publications appeared in Austria, Germany, Denmark, Sweden and England, concerning cases of 'Sulphonal poisoning'; the great majority in women and many of them lethal. The dark red urine, like port or burgundy, was characteristic. Hammarsten (1891; 1892) reported that a pigment closely resembling 'haematoporphyrin' was present in these urines. In 1890, Ranking and Pardington described two women who excreted 'haematoporphyrin' and who exhibited the gastro-intestinal and neuro-psychiatric manifestations of the disease, which is now called acute intermittent porphyria. In both of these patients there was no association of the onset of the attack with drugs.

After the introduction of barbiturates into clinical medicine in 1903, they were soon associated with the onset of acute attacks of porphyria. Dobrschansky (1906) described a case with the usual distinguishing gastro-intestinal and neuro-psychiatric symptoms of acute porphyria, occurring in a patient after prolonged administration of diethylbarbituric acid. Thirty years later, in 1937, Waldenstrom of Lund University put forward the theory that some barbiturates could precipitate latent porphyria into the acute form. It was also in this report that diseases of porphyrin metabolism were first referred to as the porphyrias rather than haematoporphyrins. In the following years, several other authors reported deterioration in the clinical and metabolic state of porphyric patients after administration of barbiturates (Eliaser and Kondo, 1942; Denny-Brown and Sciarra, 1945; Prunty, 1946; Whittaker and Whitehead, 1956). It has now been conclusively shown that barbiturates adversely affect the hepatic porphyrias, either by provoking attacks or, if taken after the beginning of an attack, by profoundly aggravating the severity of the neurological symptoms (Goldberg, 1959; Eales, 1971).

Gunther was the first to classify the diseases of porphyrin metabolism in two papers in 1911 and 1922, and it is he who is credited with the first recognition that porphyria was an 'inborn error of metabolism' by Garrod in 1923, in the second edition of his monograph. Gunther also defined and named for the first time the very rare condition, 'congenital haematoporphyrin', in which the predominating

symptom, skin photosensitivity, starts in early childhood. It was the study of one of Gunther's congenital haemato-porphyrin patients by Hans Fischer which led to the great advancement of the knowledge of porphyrin chemistry between the years 1915 and 1945. Fischer showed that the naturally occurring porphyrins of excreta, uroporphyrin and coproporphyrin, differed structurally from haematoporphyrin, which was considered to be a chemical artefact (Fischer et al., 1925; Fischer and Duesberg, 1932). The naturally-occurring porphyrin of haem was also differentiated from haematoporphyrin by a number of investigators, including Laidlaw (1904), Fischer (1915a,b) and Schumm (1924), and the name protoporphyrin was suggested for this porphyrin by Fischer. Thus, the diseases of porphyrin metabolism came to be termed Porphyrrias by Waldenstrom (1937) rather than Haemato-porphyrrias (Gunther, 1911).

B. CLASSIFICATION OF THE PORPHYRIAS.

Hans Gunther was the first to classify the diseases of porphyrin metabolism in two comprehensive papers published in 1911 and 1922. Further surveys and reclassifications were published in the following years by Waldenström (1937), Schmid et al. (1954), Waldenström (1957) and Watson (1960). The most helpful of the classifications of porphyrias was that introduced by Schmid et al. (1954). They divided the porphyrias into erythropoietic and hepatic groups, depending on whether the bone marrow or the liver respectively was the tissue in which the aberration of metabolism mainly occurred. The following classification in Table 1 will be used in this Thesis (Conference Discussion, 1963).

TABLE 1

CLASSIFICATION OF THE PORPHYRIAS

I. HEPATIC PORPHYRIAS

- 1) Acute intermittent porphyria.
- 2) Variegate porphyria.
- 3) Hereditary coproporphyria.
- 4) Cutaneous hepatic porphyrias:
 - a) hereditary type;
 - b) possibly genetically predisposed (alcoholism);
 - c) acquired (e.g. hexachlorobenzene).

II/

II. ERYTHROPOIETIC PORPHYRIA.

- 1) Congenital (erythropoietic) porphyria.
- 2) Erythropoietic protoporphyria.
- 3) Erythropoietic coproporphyria.

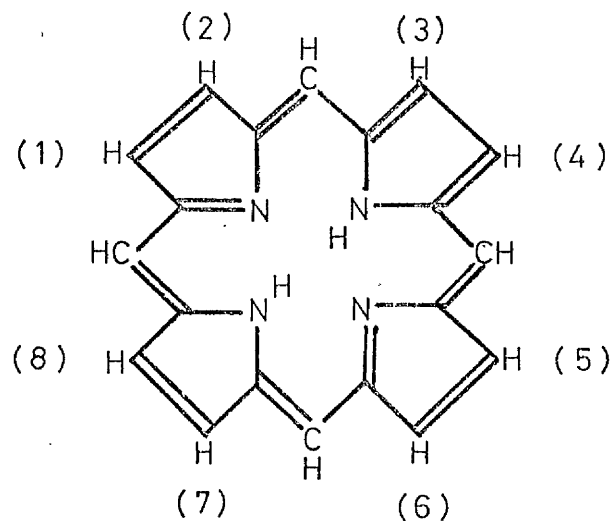
C. PORPHYRIN AND HAEM STRUCTURE AND BIOSYNTHESIS.

The porphyrins derive their name from the resemblance of their colour to porphyry, ultimately from the Greek word, porphyros, meaning crimson. They are widely distributed throughout the animal and vegetable kingdom. In the free form, the porphyrins occur in small amount but their metallo-protein complexes, for example haemoglobin, cytochromes and the chlorophylls, are quantitatively more important and play a vital role in the living cell.

The basic chemical structure (Fischer, 1927; 1930), which is the same for each porphyrin, is a macrocyclic structure. This porphin structure consists of four hetero-cyclic rings, namely one pyrrole, two pyrrolenine and one maleinimide ring, which are joined together by methene bridges (Fig. 1). The individual porphyrins are derived from porphin by substitution of the hydrogen atoms occupying the eight $\beta \beta'$ positions of the rings. Replacement of these hydrogen atoms by two dissimilar groups, for example by a methyl and ethyl group as in the aetioporphyrins, can be made in four possible ways leading to four position isomers (Fig. 2). Fischer (1927; 1930; 1937) synthesised all four aetioporphyrins and proposed that all porphyrins should be referred to them for designation of isomeric type. There are thus four possible coproporphyrins (substituent groups, $-\text{CH}_3$ and $-\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$) and four possible uroporphyrins (substituent groups, $-\text{CH}_2\cdot\text{COOH}$ and $-\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$). In the protoporphyrins, the $\beta \beta'$ hydrogen atoms are replaced by four methyl, two vinyl and two propionic acid residues. This

Figure 1.

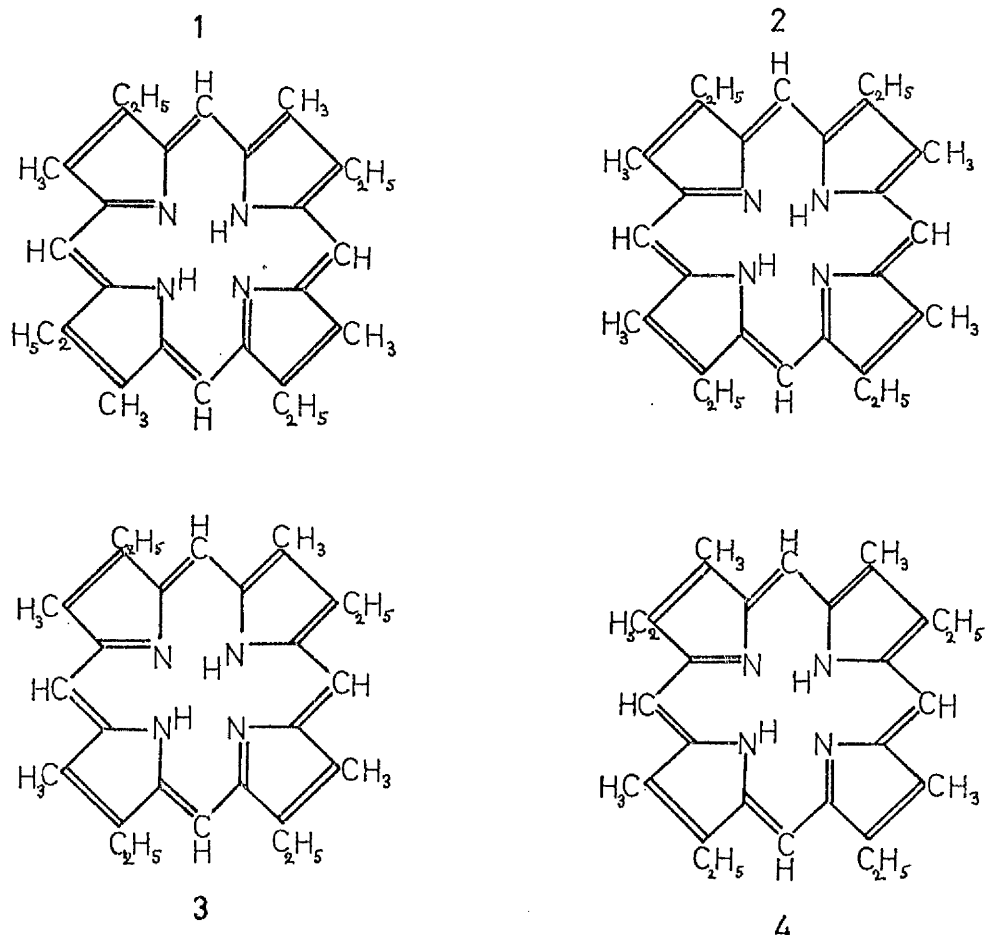
THE BASIC CHEMICAL STRUCTURE OF THE PORPHYRINS



The eight $\beta\beta'$ positions of the rings are numbered

Figure 2.

THE FOUR ISOMERIC AETIOPORPHYRINS



gives rise to fifteen possible isomeric forms; but, so far, only the single protoporphyrin IX (Fig. 3) has been encountered in nature, either in the free state or as an iron complex, haem (Fig. 4). Similarly, only coproporphyrin I and III and uroporphyrins I and III have so far been found to occur in nature.

THE HAEM BIOSYNTHETIC PATHWAY.

Several good reviews of this work have been published (Shemin, 1956a,b; Rimington, 1956, 1958, 1959a; Stich, 1959; Shemin, 1970). From these studies, the general pathway of haem biosynthesis may be regarded as firmly established.

Haem synthesis is initiated by the condensation of glycine and succinyl CoA in the presence of ALA synthetase (ALA.S) (Shemin and Kumin, 1952) (Fig. 5). The co-factors for this reaction are pyridoxal phosphate (Schulman and Richert, 1956) and ferrous iron (Brown, 1958). The pyridoxal phosphate activates the methylene group of glycine, preliminary to Schiff base formation. This glycine and activated succinate, succinyl CoA, then condense to form α -amino- β -keto-adipic acid which, being very unstable, readily decarboxylates to form δ -aminolaevulinic acid (ALA). ALA synthetase appears to be rate limiting in the pathway (Lascelles, 1964; Levere and Granick, 1965), and hence its regulation can determine the rate of hepatic haem formation from glycine and succinyl CoA (Granick and Levere, 1968). This enzyme has a relatively short half-life; one hour in mammalian systems (Marver et al., 1966b; Stein et al., 1970) and four to six hours in avian

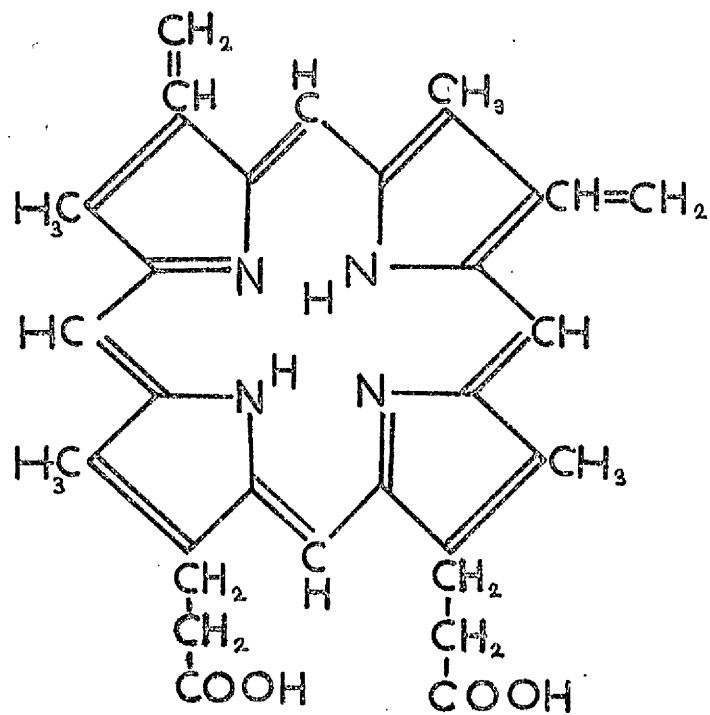
PROTOPORPHYRIN IX

Figure 4.

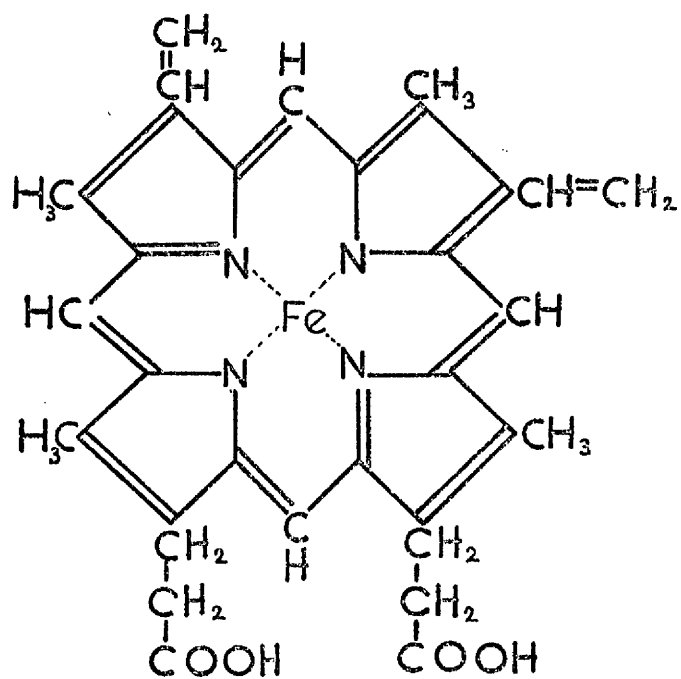
HAEM

Figure 5.

ALA SYNTHETASE
(Succinyl CoA : Glycine, Succinyltransferase)

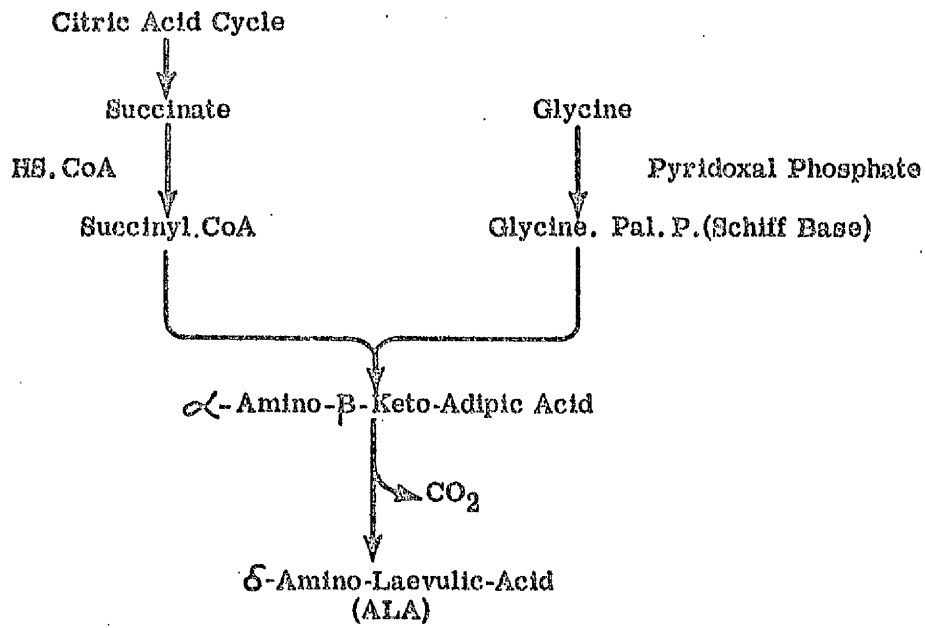
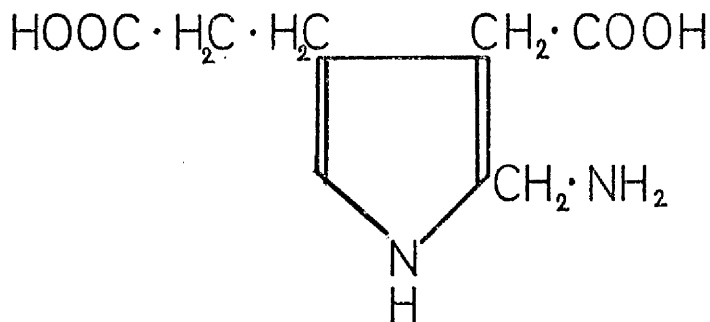


Figure 6.

Porphobilinogen



systems (Granick, 1966). Recent studies provide evidence that haem is involved in the negative feed-back regulation of ALA.S, both by inhibiting the activity of the enzyme (Burnham and Lascelles, 1963; Scholnick et al., 1969) and participating in the repression of its synthesis (Hayashi et al., 1968; Marver, 1969; Sassa and Granick, 1970). With respect to the former mechanism, haem, the end-product of the biosynthetic chain, would by binding to an allosteric site on ALA.S directly inhibit its enzymic activity. This mechanism has been clearly shown to operate in *R. spheroides* (Lascelles, 1960). This inhibition is reversible by the removal of the added haem. Since both ALA.S and the enzyme ferrochelatase, which forms haem from protoporphyrin, are localized in mitochondria, one might suppose on teleological grounds that this mechanism would permit control of ALA.S activity in mammalian cells as well. Evidence in support of this possibility has been presented by Karibian and London (1965), who demonstrated that haemin added to iron-deficient rabbit reticulocytes inhibited by 50 per cent the incorporation of labelled glycine into the haem of haemoglobin; whereas ALA incorporation into haem was not significantly affected. In contrast to this, the increased ALA.S of mitochondria isolated from induced chick embryo liver cells is not inhibited by added haem (Granick, 1966). Similarly, Vavra (1967) did not observe such inhibition by haem in mitochondria derived from chicken reticulocytes.

The alternative mechanism for haem control of ALA.S,

i.e. end-product repression, has been demonstrated to be operative in both bacterial and animal species. In *R. spheroides*, Burnham and Lascelles (1963) showed that haem can decrease by 60-80 per cent the induced formation of ALA.S. A similar demonstration of haem repression of ALA.S formation has been made in embryonic chick liver cells growing in primary culture (Granick, 1966). Kappas and Granick (1968) demonstrated that haem represses maximally when administered to hepatic cells at the same time as the enzyme inducers, but represses only weakly if added at a time when the induction process is well advanced. This effect is similar to that observed with inhibitors of nucleic acid synthesis, such as Actinomycin D. (Kappas et al., 1968). This time relationship of the haem blocking effect on porphyrin formation is good evidence that haem enters the cell but does not act by inhibition of the activity of the enzyme but acts rather at some early event in the induction process; possibly by repressing activity of the structural gene which codes for ALA synthetase. An in vivo study in the rat, showing results consistent with haem repression of induced synthesis of ALA.S, has also been reported by Waxman et al. (1966). More recent studies by Sassa and Granick (1970) give evidence that haemin inhibits the synthesis of ALA.S in chick embryo liver cells in culture at the translational, rather than at the transcriptional level as had been previously hypothesized (Granick, 1966). They now

suggest that haemin might inhibit by competing with an inducing chemical, by acting at some other site, or by causing a decrease in the lifetime of mRNA for ALA synthetase. This latter idea is disfavoured by Sassa and Granick (1970), as they noted that haemin did not stimulate an increase in ribonuclease activity.

After the formation of δ -aminolaevulinic acid by ALA.S, the next step of the haem biosynthetic pathway is the condensation of two molecules of ALA with elimination of water, by the enzyme ALA dehydrase (ALA.D). This enzyme was first described by Dresel and Falk (1953) and then by Gibson, Neuberger and Scott (1955). This condensation forms the monopyrrole precursor of the porphyrins, porphobilinogen (PBG), which was first isolated from porphyria urine by Westall in 1952, and its structure (Fig. 6) determined by Cookson and Rimington (1953; 1954).

The subsequent steps of this pathway involve formation of the tetrapyrrole porphyrin, uroporphyrinogen, from four units of PBG, followed by the successive decarboxylation of the octacarboxylic porphyrinogen thus formed, to dicarboxylic porphyrin, protoporphyrin IX. The initial step of this sequence is catalysed by URO-I-synthetase (PBG deaminase). Bogorad (1958a,b) and Cornford (1964) have suggested that the mechanism of this reaction is firstly a condensation of the porphobilinogen monomer units into a polymer of intermediate length, followed by a cyclisation of this polymer into uroporphyrinogen I units. If the enzyme, URO-III-cosynthetase

(uroporphyrinogen isomerase), is present, this reaction proceeds with the production of the biologically viable series III isomer. If this enzyme is absent, it produces the series I isomer. In natural systems, the series I isomer cannot be converted into protoporphyrin and will only produce uroporphyrin I and coproporphyrin I, which are excreted in the urine. It has been reported that in congenital erythropoietic porphyria (Borst and Konigsdorffin, 1929) and in some cases of porphyria cutanea tarda (Pathak and Burnett, 1964) excretion and accumulation of these series I porphyrins, especially uroporphyrin I, takes place in the skin. The partial absence of the URO-III-cosynthetase is a possible explanation for these observations (Lockwood and Benson, 1960; Magnus, 1968).

Coproporphyrinogen, the subsequent substance on the pathway, is produced by the successive decarboxylation of uroporphyrinogen by uroporphyrinogen decarboxylase. In this sequence of reactions, the series III isomer is decarboxylated twice as rapidly as the series I isomer (Mauzerall and Granick, 1958).

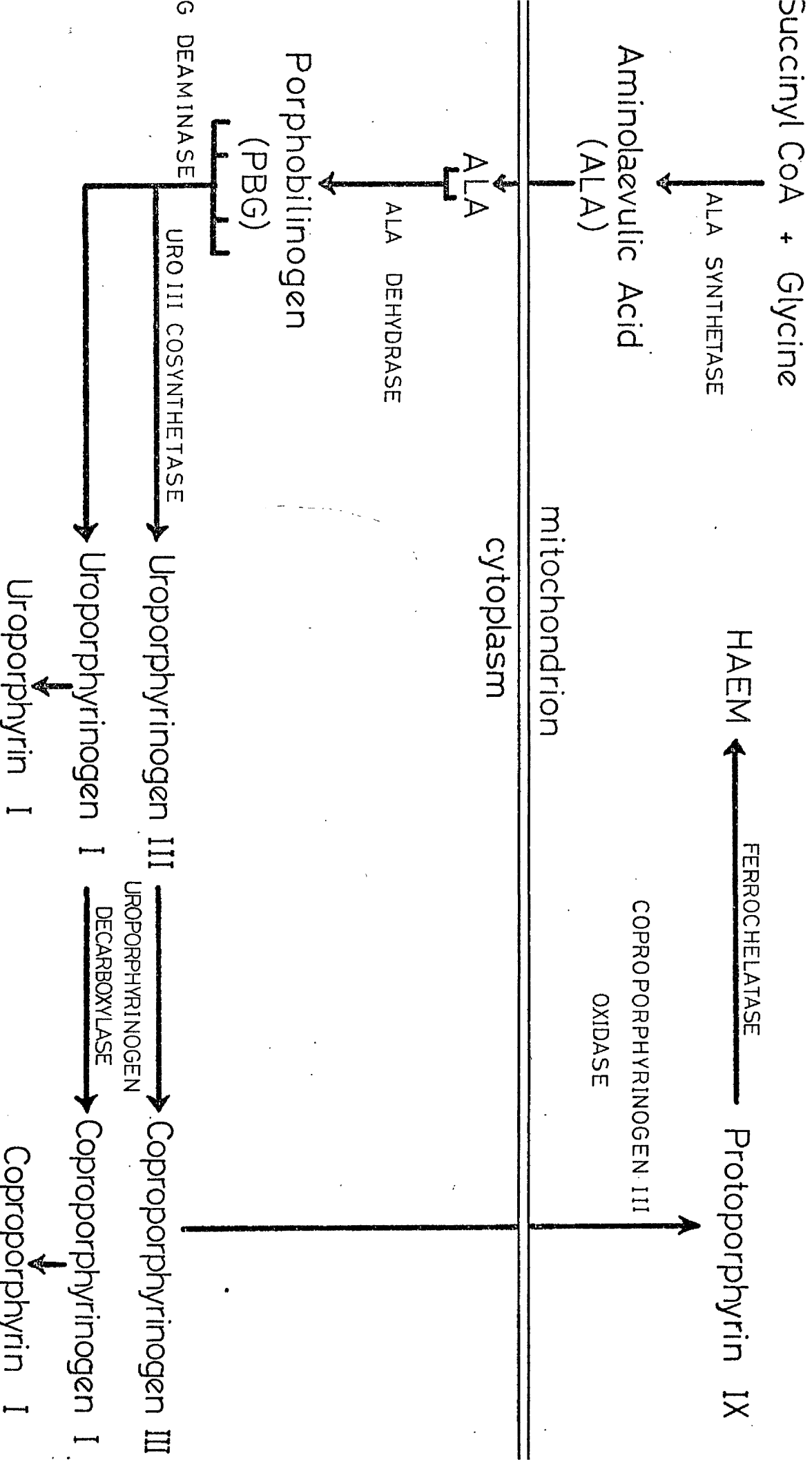
By the action of the mitochondrial enzyme, coproporphyrinogen oxidase and decarboxylase, the tetracarboxylic coproporphyrinogen III is converted to protoporphyrin IX through the intermediate tricarboxylic and dicarboxylic porphyrinogen stages. It is these enzymes that cannot handle the series I isomer (Sano and Granick, 1961; Batlle et al., 1965).

The final enzyme of the pathway is haem synthetase

(ferrochelatase) (Goldberg et al., 1956; Labbe and Hubbard, 1960; Porra and Jones, 1963). This enzyme catalyses the insertion of ferrous iron into protoporphyrin to form the important substance haem. The complete pathway (Fig. 7) is reviewed in detail by Goldberg and Rimington (1962).

Figure 7.

THE HAEM BIOSYNTHETIC PATHWAY.



SECTION II

THE INDIVIDUAL PORPHYRIAS

THE INDIVIDUAL HEPATIC AND ERYTHROPOIETIC PORPHYRIAS.

The porphyrias are metabolic disorders, mostly of hereditary nature, characterized by increased excretion of porphyrins and their precursors, which accumulate in large excess within the body. In the porphyrias, the synthesis of these pigments is greatly increased on account of a failure of the control mechanisms that normally regulate the synthesis of haem. These disturbances should be regarded as disorders of the regulation of haem synthesis.

The normal urine usually contains trace amounts of uroporphyrin, coproporphyrin, ALA and PBG. Both the faeces and blood normally contain small amounts of coproporphyrin and protoporphyrin. It has also been shown by French and Thonger (1966) that coproporphyrin and protoporphyrin are present in bile and meconium.

In the porphyrias, any of these porphyrins or their precursors may be over-produced and excreted in excess. The two main tissues in which this aberration may occur are the bone marrow and the liver. This fact is used to divide the porphyrias into two major groups, the erythropoietic and the hepatic, according to whether the site of the disorder of haem synthesis is in the bone marrow or the liver, respectively.

A. THE ERYTHROPOIETIC PORPHYRIAS.

Congenital (erythropoietic) porphyria: This is one of the rarest of the porphyric group of diseases. There are now less than a hundred recorded cases with approximately equal numbers of males and females (Schmid, 1966; Kaufman et al., 1967).

The disease is characterized by marked photosensitivity and by increased excretion of uroporphyrin and coproporphyrin, predominantly of the unphysiological type I isomer. The bones and teeth are also discoloured reddish-brown because of their porphyrin content. About one-quarter of the reported patients had an onset from birth, and in all cases the condition was revealed before the sixth year (Schmid et al., 1955). The available data from the studies and reviews of this disease (Gray, 1925, 1926; Schmid et al., 1955; Watson et al., 1958) are compatible with, but not proof of a Mendelian recessive heredity (Schmid, 1966; Romeo et al., 1970). This view is strengthened by the definite genetic studies in bovine congenital porphyria, which closely resembles the human disease, by Fourie (1936, 1939) and Amoroso et al. (1957).

The nature of the genetic abnormality is not yet determined. Taddeini and Watson (1968) assert that there is clearly induction of ALA-S in the young red cells of the bone marrow because of the marked overall increase of porphyrins, including uroporphyrin I and protoporphyrin IX. Lockwood and Benson (1960) proposed that a partial absence of URO-III-co-synthetase, which converts uroporphyrinogen I to uroporphyrinogen III,

could explain the accumulation of the physiologically ineffective uroporphyrin I in this condition. This hypothesis does not explain the actual increase in the overall formation of series III porphyrin which exists in this disease (Taddeini and Watson, 1968). To overcome this, Kramer et al. (1965) have suggested a possible alternate mechanism; namely, a lack of suppressor of URO-I-synthetase (PBG deaminase) with overproduction of type I isomer, in addition to normal amounts of type III isomer.

Erythropoietic protoporphyria: This is the most common of the erythropoietic porphyrias. The main biochemical feature is greatly increased amounts of protoporphyrin and, to a lesser extent, coproporphyrin in the erythrocytes. This may be accompanied by excessive quantities of coproporphyrin and protoporphyrin in the faeces. In the majority of cases, the urine has a normal content of porphyrins (Magnus et al., 1961). Haeger-Aronsen (1963) has shown that in one family the disease has been transmitted by a Mendelian dominant gene. Further reports by Snitker and Lintrup (1967) and Haeger-Aronsen and Krook (1965) are in agreement with this observation.

The disease is characterized clinically by the onset of photosensitivity, usually commencing in the first two years of life and persisting throughout the patient's lifetime. The incidence of this condition is at present unknown, but it is probably not greater than two per cent of all causes of photosensitisation (Prentice and Goldberg, 1969).

The site of the chief lesion in erythropoietic protoporphyria

is not yet known, and the evidence that it is erythropoietic rather than hepatic is contradictory. Examination of the bone marrow smears under ultra-violet light reveals numerous red fluorescent normoblasts (Magnus et al., 1961). Prentice and Goldberg (1967) also reported fluorescence in the marrow in this disease but Runge, cited by Watson (1966), failed to demonstrate it in the marrow normoblasts from any of three patients with this disease. Kaplowitz et al. (1968) photohaemolyzed blood cells from patients with erythropoietic protoporphyria and isolated photoresistant erythrocytes. Two populations of cells appeared to be present; one protoporphyrin rich and photosensitive, and the other with a normal protoporphyrin level. Similar observations were presented by Schothorst et al. (1970), who found that the concentration of protoporphyrin in upper layers of centrifuged blood was greater than in lower layers.

There is also evidence that the liver in this condition forms excessive porphyrins and might be responsible for the increased porphyrins in the plasma and the faeces (Redeker et al., 1963). The occasional occurrence of cirrhosis (Gajdos and Gajdos, 1969; Gray, 1970) and the path of incorporation of labelled glycine into faecal porphyrins and stercobilin in erythropoietic protoporphyria, supplies further evidence that there is also a hepatic defect of porphyrin biosynthesis (Gray et al., 1964; Redeker and Bronow, 1964). In 1968, Redeker and Sterling demonstrated that the faecal and plasma porphyrins increased markedly with fasting and decreased

with a rich provision of glucose. This suggests a hepatic source of the faecal and plasma porphyrins, since the liver has been the only site at which the glucose effect (catabolic repression) has been shown to be operative (Loomis and Magasanik, 1964). A significant increase in hepatic ALA.S in two patients with erythropoietic protoporphyria has been reported by Miyagi in 1967. In more recent investigations into erythropoietic protoporphyria, Schwartz and his co-workers (1971) reported that in vitro data indicated a 30-fold increase in ALA.S activity in the patient's blood, with a relative deficiency of haem synthetase activity. They also showed by isotopic studies the presence of several pools of erythrocyte protoporphyrin, each with markedly different turnover rates. These and other findings suggest that the most significant defects in this disease reside in the reticulocyte and late normoblast stages of erythroid development.

Congenital erythropoietic coproporphyria: This third type of erythropoietic porphyria was described by Heilmeyer and Clotten in 1964. Clinically, it is characterized by skin photosensitivity. In this porphyria, the urine and the faeces had a normal porphyrin content but the red cells contained excessive quantities of coproporphyrinogen series III isomer. The same biochemical disturbance was also found in the mother of the patient, and the authors have suggested that this disease is transmitted as a Mendelian dominant character. No other patients with this disease have so far been reported.

There is no concrete evidence that these three disorders of porphyrin metabolism in the bone marrow can be affected by sedatives and by the other drugs that can influence the picture of hepatic porphyrias.

B. HEPATIC PORPHYRIAS.

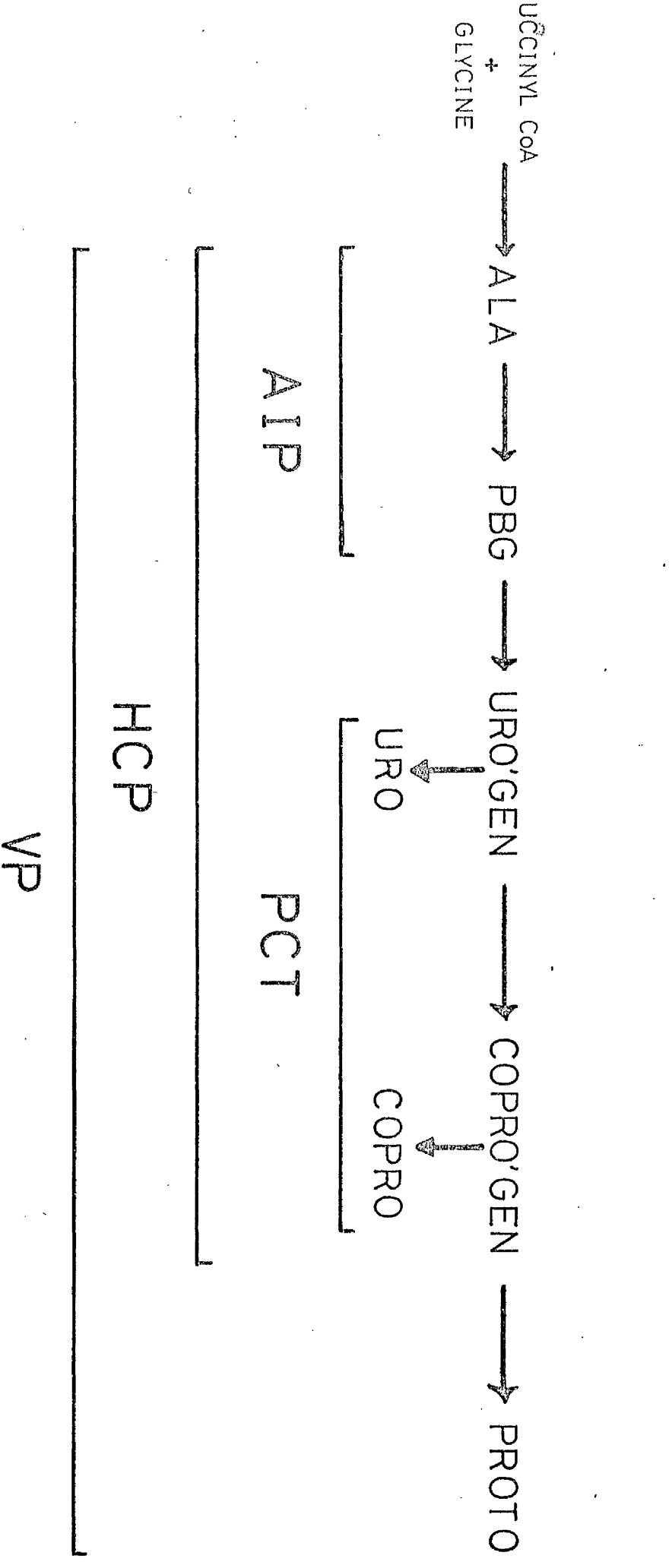
Introduction.

The hepatic porphyrias are a group of disorders characterized by excessive levels of porphyrins and their precursors in the liver. This group is more common than the erythropoietic porphyrias. They are classified on the basis of clinical manifestations and unique patterns of porphyrin and porphyrin precursors excretion (Fig. 8) (Waldenström, 1957; Schmid, 1966). The defect responsible for each of these diseases is inherited as a Mendelian autosomal dominant character (Goldberg et al., 1967), with the exception of porphyria cutanea tarda which appears in most cases to be acquired (Eales, 1961).

The hepatic porphyrias can be designated into four main groups, which are - (1) acute intermittent porphyria (latent and manifest), in which the skin is not photosensitive, but intermittent abdominal pain and neuropsychiatric manifestations are the main clinical symptoms; (2) variegate porphyria (the genetic type of porphyria of South Africa), in which there is a combination of photosensitivity with manifestations of acute intermittent porphyria within the same family and sometimes within the same individual; (3) the cutaneous hepatic porphyrias (porphyria cutanea tarda, porphyria cutanea tarda hereditaria, various acquired porphyrias), in which skin photosensitivity

Figure 8.

Porphyrins and precursors excessively excreted during the acute phases
of each of the hepatic porphyrias



is the predominant clinical feature; (4) hereditary coproporphyria, which usually presents in the same way as AIP, although a few patients with this condition have presented photosensitivity.

Acute Intermittent Porphyria.

There have been several comprehensive clinical reviews on this subject (Gunther, 1911, 1922; Waldenström, 1937; Goldberg and Rimington, 1962). It is clinically distinguishable from the other hepatic porphyrias by the dominance of gastro-intestinal and neuro-psychiatric symptoms and the absence of photosensitivity. The most important symptoms are abdominal pain, vomiting and constipation with, less commonly, paralysis or paresis and psychological manifestations of various types. The diagnosis of this condition is usually made by the detection in the urine of excessive quantities of ALA and PBG. It has been established that patients have in their liver a marked increase in the enzyme ALA.S (Tschudy et al., 1965).

The main characteristic of this disease is its intermittency. The cause of the intermittent attacks has been partly ascribed to the administration of drugs such as barbiturates (Waldenström, 1939, 1940; With, 1957; Goldberg, 1959; Schmid, 1966; Eales, 1971), alcohol (Goldberg and Rimington, 1962; Ferguson et al., 1970), certain steroids such as are contained in contraceptive

pills (Wetterberg, 1964; Rimington and De Matteis, 1965), pregnancy (Nielson and Nielson, 1958) and a diet low in carbohydrate and protein (Welland et al., 1964; Felsher and Redeker, 1967; Knudsen et al., 1967). In 1954, Goldberg demonstrated that out of nine barbiturates given to rabbits, six had a significant effect on the urinary excretion of coproporphyrin. These same nine barbiturates all caused a significant elevation of hepatic ALA.S in rats (Moore et al., 1970).

There is now ample evidence that oral contraceptives can provoke attacks of acute intermittent porphyria (Wetterberg, 1964; Rimington and De Matteis, 1965; Redeker, 1965) and also of variegate porphyria (Watson et al., 1962). In contrast to this, there have been some reports of the use of these combined contraceptive preparations to alleviate the symptoms of porphyria in some female patients in whom the attacks of porphyria could be associated with pregnancy or onset of menses (Haeger-Aronsen, 1963b; Welland et al., 1964; Perlroth et al., 1965).

The effect of diet has also been stressed by Welland et al. (1964). A diminution of carbohydrate and protein intake may provoke an acute attack. Knudsen and his co-workers (1967) reported an 18-year old patient who was provoked into a severe attack when placed on a water diet for obesity. A similar case has also been noted by Felsher and Redeker (1967).

Porphyria Variegata.

This porphyria was so named because it could present itself in a variety of ways, with skin lesions or with an acute attack, or with both these states. It has been described in Sweden (Hamnström et al., 1967), in the U.S.A. (Taddeini and Watson, 1968), in the United Kingdom (Rimington et al., 1968; Cochrane and Goldberg, 1968) and in South Africa by Dean and Barnes (1959). The latter two investigators stated that there is a prevalence rate of three in one thousand of the white population in South Africa. This type of porphyria in white South Africans is apparently inherited as a Mendelian dominant character (Dean and Barnes, 1955).

The symptoms of the acute attack are very similar to those of AIP but variegate porphyria differs from AIP in two main respects: 1) cutaneous manifestations in the form of photosensitivity and increased skin fragility can occur with acute attacks in the same patient, or in other members of the patient's family; 2) there is a high excretion of faecal coproporphyrin and faecal protoporphyrin in states of remission (with an increase in the urinary coproporphyrin, PBG and ALA during the acute attacks, but which often returns to normal or near normal during quiescence (Dean, 1963)).

Bloch (1965) concluded from his study of pregnancy in sufferers from porphyria variegata that the disease has no effect on pregnancy nor does pregnancy adversely affect the

disease. The investigations of Goldswain and Eales (1971) seem to be in agreement with this. In 1965, Dean reported a patient with porphyria variegata who was adversely affected by the contraceptive pill. In the large study of patients with variegate porphyria by Eales (1971), oral contraceptives were found to be associated with acute attacks in four patients but in each case other drugs had also been administered. In this forementioned publication, Eales reports the drugs and other factors involved in the induction or potentiation of 145 acute attacks in 120 patients, 107 of whom were VPs and the rest AIPs. The cardinal role of the barbiturates in the genesis of the severe attack in AIP and VP is emphasised by this report that over 50% of the acute attacks were associated with barbiturate medication and in more than 20% of attacks they were the only drug involved.

Dowdle et al. (1967) and Masuya (1969) have demonstrated excessive ALA.S activity in the livers of patients with VP. In further metabolic studies with labelled ALA in normal and porphyric human subjects including two patients with porphyria variegata, Dowdle et al. (1968) concluded that excessive ALA synthetase activity is common to AIP, VP and symptomatic porphyria. Similar investigations were undertaken by Strand et al. (1971), who determined the hepatic activity of ALA.S and uroporphyrinogen I synthetase (URO-I-synthetase) in specimens of liver from normal and porphyric subjects. These workers reported that ALA.S activity was markedly increased

in three patients with AIP and two with VP, but was normal in four patients with porphyria cutanea tarda. The studies of URO-I-synthetase revealed that in AIP the activity of this enzyme was less than 50% of the controls, in contrast to the normal values of the patients with other forms of porphyria. Taking into consideration the excretory pattern of AIP, Strand and his co-workers suggested that these results favour a primary defect at the level of the enzymatic conversion of PBG to uroporphyrinogen by URO-I-synthetase, which secondarily leads to induction of ALA synthetase. If AIP is a consequence of a gene defect affecting enzymatic conversion of PBG to porphyrins, it would appear likely that the enzymatic differences characterizing each of the other genetically transmitted hepatic porphyrias result from independent mutations.

Hereditary Coproporphyria.

The salient biochemical abnormality of this type of hepatic porphyria is the excessive excretion of coproporphyrin III in faeces and urine at all times. The condition may remain symptomless, but it may be provoked by drugs such as barbiturates and sulphonamides (McIntyre et al., 1971), oestrogens and pregnancy, into acute attacks resembling those of AIP and VP. The patients described by Goldberg et al. (1967), Cannon and Turkington (1968), Dean et al. (1969) and Hunter et al. (1971) developed acute manifestations during pregnancy. This evidence suggests the involvement of endocrine substances in the episodic

exacerbations of this disease in certain patients. During the acute attacks, there is an excessive excretion of PBG and ALA in the urine. Despite these similarities to AIP and VP, the three hereditary hepatic porphyrias can be distinguished by the pattern of faecal porphyrin excretion. In HCP, large amounts of coproporphyrin are constantly present in the faeces; in VP, faecal excretion of both coproporphyrin and protoporphyrin is excessive, while in AIP there is only a slight increase in excretion of both of these substances.

There is evidence that, as with AIP and VP (Tschudy et al., 1965; Nakao et al., 1966; Dowdle et al., 1967; Masuya, 1969), the activity of hepatic ALA.S is increased during an acute attack (Kaufman and Marver, 1970; McIntyre et al., 1971). The inheritance pattern of hereditary coproporphyria strongly suggests that it is transmitted as a non-sex-linked Mendelian dominant character (Goldberg et al., 1967; Haeger-Aronsen et al., 1968). In this last mentioned publication, the authors put forward the hypothesis that HCP is caused primarily by a structural defect in the mitochondrion, either in wall structural proteins or in respiratory chain enzyme activity; i.e. a deficiency in coproporphyrinogen oxidase. A similar hypothesis was proposed by Kaufman and Marver (1970), who suggested that the primary abnormality in HCP, AIP and VP is a partial block in haem biosynthesis, the block being at a different point in the pathway in each of the three diseases. In

the case of HCP, the defect would be in the conversion of coproporphyrinogen III to protoporphyrinogen by the enzyme coproporphyrinogen oxidase. Since haem inhibits the synthesis of ALA.S, an increase in enzyme activity would result if free haem levels dropped due to the block in haem synthesis. Secondary elevation of ALA.S could account for the increase in ALA and PBG excretion which occurs in all three diseases, while the site of the block would explain the differing patterns of porphyrin excretion.

Cutaneous Hepatic Porphyria.

The group of cutaneous porphyrias which are of hepatic origin have been named 'cutaneous hepatic porphyrias' (Goldberg and Rimington, 1962). They represent a very heterogeneous group of diseases and include a number of conditions usually considered under the name of symptomatic porphyria. They may result from many different aetiological factors such as, an hereditary defect of the porphyrin metabolism of the liver (Tio, 1956; Waldenström, 1957; Holti et al., 1958); a variety of hepatotoxic processes including alcohol (Canivet and Rimington, 1953; Bolgert et al., 1953; Prato, 1964); liver poisons such as hexachlorobenzene (Cetingil and Özen, 1960; Schmid, 1960a) or a simple neoplasm of the liver (Tio et al., 1957).

Prolonged intake of adulterated alcohol is thought to be the causative factor of the cutaneous hepatic porphyria observed in the Bantus of South Africa (Eales, 1963). As

only a small proportion of chronic alcoholics and patients with liver disease develops symptoms of cutaneous porphyria, the existence of an underlying genetic disposition is suspected but, with a few possible exceptions (Waldenström and Haeger-Aronsen, 1963; Prato and Zina, 1965), it has not been conclusively demonstrated. In contrast to this, the toxic porphyria brought about by hexachlorobenzene is a clearly acquired condition occurring without a genetic disposition to porphyria. In 1956, an outbreak of cutaneous porphyria, involving several thousand persons from three different ethnic groups, was reported in South Eastern Turkey. Affected persons, predominantly children, exhibited photosensitivity, hepatomegaly and marked porphyrinuria, but no abdominal or neurological symptoms (Cam, 1959; Schmid, 1960; Cetingil and Özen, 1960). Poisoning by hexachlorobenzene, a fungicidal agent introduced in bread made from dressed wheat intended for sowing, was suspected as the cause of these disturbances. This suspicion was soon confirmed by the finding that hexachlorobenzene could cause a condition of porphyria when fed to the rat and rabbit (De Matteis et al., 1961; Ockner and Schmid, 1961). In 1959, the treatment of wheat with hexachlorobenzene was discontinued in Turkey and, after this measure, no new cases were reported (Cam and Nigogosyan, 1963).

Several cases have also been described in which a marked increase in porphyrin excretion and the appearance of clinical symptoms of photosensitivity have been observed in association with the administration of certain hormonal

preparations (Warin, 1963; Becker, 1965; Copeman et al., 1966; Felsher and Redeker, 1966; Zimmerman et al., 1966; Thivolet et al., 1967; Vail, 1967). The majority of these cases were elderly male patients receiving oestrogen preparations for treatment of prostate carcinoma, and an interval of several months or years had elapsed between the starting of the therapy and the appearance of the cutaneous symptoms. Withdrawal of oestrogens led to a gradual improvement in the cutaneous symptoms (Zimmerman et al., 1966), although porphyrin excretion remained elevated in all six patients in which it was followed (Warin, 1963; Becker, 1965; Copeman et al., 1966; Zimmerman et al., 1966).

In all these cutaneous hepatic porphyrias, the liver forms excessive porphyrins which are normally excreted in the bile and pass into the faeces (Rimington, 1959b). As long as the excretion route is efficient, symptoms are unlikely, but in the event of hepatic dysfunction the excessive porphyrins are deviated to the systemic circulation and are then excreted, mainly in the urine but also in the skin (Schück and Berman, 1956). As a result of this deviation of the porphyrins, the main skin manifestations occur; namely, photosensitisation, excessive skin fragility, hyperpigmentation and hypertrichosis. In this group of diseases there are no fluorescing normoblasts in the bone marrow (Schmid et al., 1954), and the erythrocyte protoporphyrin is normal (MacGregor et al., 1952). The plasma may contain excess porphyrins fluorescing in ultraviolet light (MacGregor et al., 1952; Wells and Rimington, 1953; Tio et al., 1957).

Moore et al. (1972a) has reported the excessive excretion of X porphyrin in the faeces of patients with PCT. This porphyrin-peptide complex has previously been reported by Rimington et al. (1968) to be elevated in VP, slightly elevated in AIP and HCP, and normal in erythropoietic protoporphyria.

In addition to the cutaneous symptoms, manifestations characteristic of AIP may be present, particularly in these forms which are hereditary (Watson, 1951; Calvy et al., 1951; Schmid et al., 1954). This may be related to the fact that the liver is the seat of the disordered porphyrin metabolism and, as in other forms of hereditary hepatic porphyria, hepatic ALA synthetase activity is elevated (Levere, 1966; Dowdle et al., 1967). There is some disagreement about this observation, as Zail and Joubert (1968), Kaufman and Marver (1970) and Strand et al. (1971) have reported that in their investigations they have found the activity of ALA synthetase to be within the normal range. In contrast to this, the results obtained by Moore et al. (1972b) demonstrate that hepatic ALA.S activity is elevated at all times in acquired PCT, as compared with normal activities. They further showed that, after treatment by venesection, this activity is lowered but not to normal levels. This lowering of activity is associated with a depression of porphyrin excretion, both in urine and faeces, and a large lowering of the serum iron saturation.

Taddeini and Watson (1968) have suggested that the various peculiarities in cutaneous hepatic porphyria in terms of

chemical sensitivity point to a constitutional or occult genetic abnormality of the liver cells, possibly microsomal in location with diminished activity of drug metabolising enzymes. They suggested that failure of the constitutionally inferior liver cell to dispose of alcohol or oestrogen might account for induction of ALA synthetase by these compounds.

SECTION III

THE STEROIDS AND PORPHYRIA

STEROIDS AND PORPHYRIA.

The association of the acute porphyric attack with the adrenal gland and hormones first began in 1889 with Urquhart's suggestion that adrenal extracts might be administered to patients with porphyria, since diseases of the adrenals had been observed in this condition. More recently, in 1947, Abrahams et al., and Linder, suggested that the clinical state of hypotension with low serum sodium and chlorides, which was observed in many cases of acute porphyria, might be due to adrenal insufficiency. Evidence of adrenal cortex hyperplasia in three patients with porphyria was reported by Prunty in 1949. A year later, Rawlings (1950) reported the case of a young woman suffering from acute porphyria who showed remarkable clinical improvement on becoming pregnant. Adrenocorticotrophic hormone (Goldberg et al., 1952) and cortisone (Watson, 1954) have been used in the treatment of porphyria with variable success. Further suggestions of hypothalamic involvement in acute hepatic porphyria were made by Hellman et al. (1962) and by Ludwig and Goldberg (1963). The studies of Tschudy (1968) demonstrate that excessive levels of antidiuretic hormone (ADH) in circulation and inappropriate release of growth hormone occur frequently in AIP. This evidence further substantiates the involvement of the hypothalamus with acute hepatic porphyria.

There are many clinical observations which suggest that certain endocrine secretions, specifically steroids, may be implicated in some way in the pathogenesis of this

group of diseases in man. Levit et al. (1957), Watson (1960) and Zimmerman et al. (1966) have summarized the clinical characteristics of the hepatic porphyrias which suggests the deleterious interplay of endocrine factors in the exacerbation of these diseases. The acute attacks occur predominantly in the third and fourth decades, when steroid excretion and production is at its peak (Fig. 9a,b); they have a marked female preponderance; they rarely become manifest clinically or biochemically before puberty, when steroid levels are low (Fig. 10); they are infrequent after the menopause, when once again steroid levels in the blood fall to lower levels (Fig. 11). As far back as 1937, Waldenström noted an apparent relation of the onset of clinical symptoms to menstruation. The cyclic exacerbation of acute (neurologic) symptoms in association with a phase of the menstrual cycle has been noted by Corrol et al. (1942), Grossfield (1951), Levit et al. (1957), Kelenyi et al. (1960), Jutzler et al. (1964), Perlroth et al. (1965) and Zimmerman et al. (1966). Kelenyi et al. (1960) noted an increase of urinary PBG with the cyclic premenstrual symptoms in their patient. Zimmerman et al. (1966) reported that, in their patient who had striking association of attacks with the luteal phase of the cycle, the excretion of PBG and porphyrin remained remarkably constant throughout the first cycle but appeared significantly increased in the second. These reports of cyclic appearance of symptoms in association with the menses suggest a hormonal relationship, but the nature

Figure 9a.

URINARY 17-OXOSTEROID EXCRETION in NORMAL HUMANS

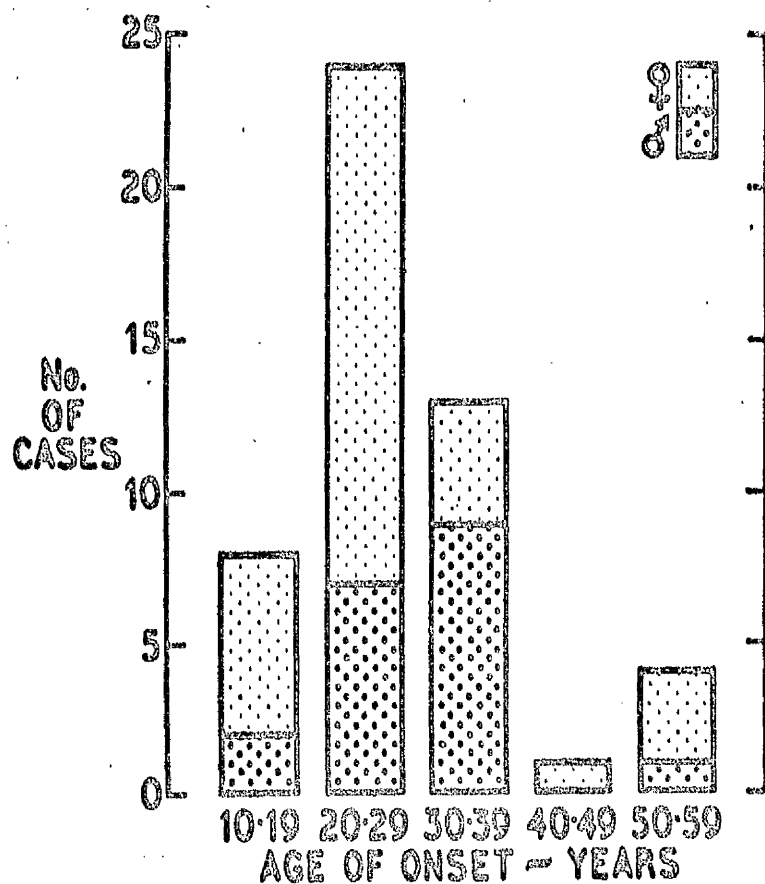
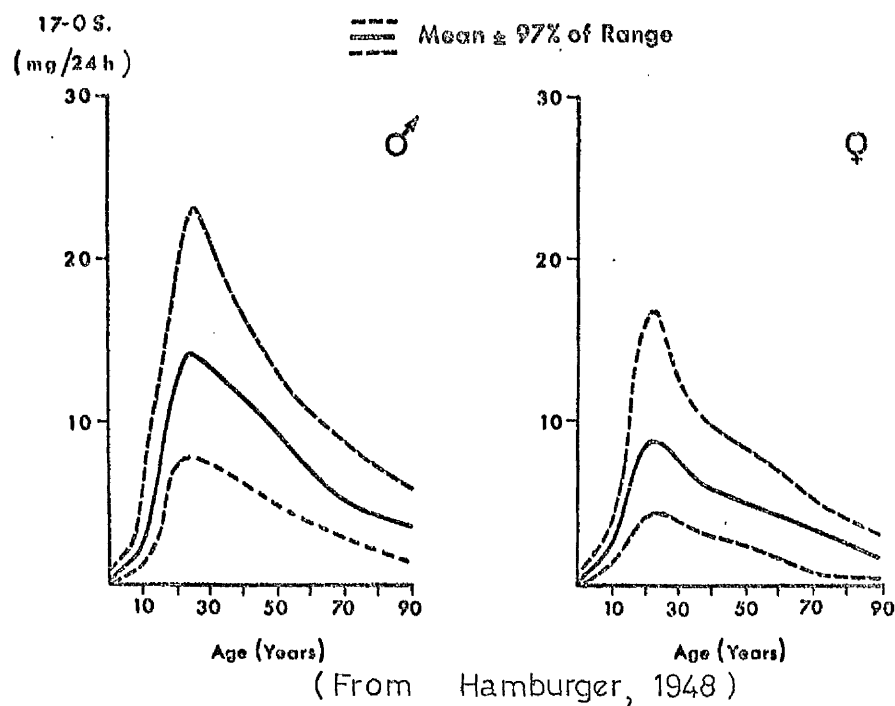


Fig. 9b. The Age of onset of attacks of porphyria in human males and females. (From Goldberg & Rimington, 1962)

Plasma levels of individual 17-oxosteroid sulphates

Fig. 10a

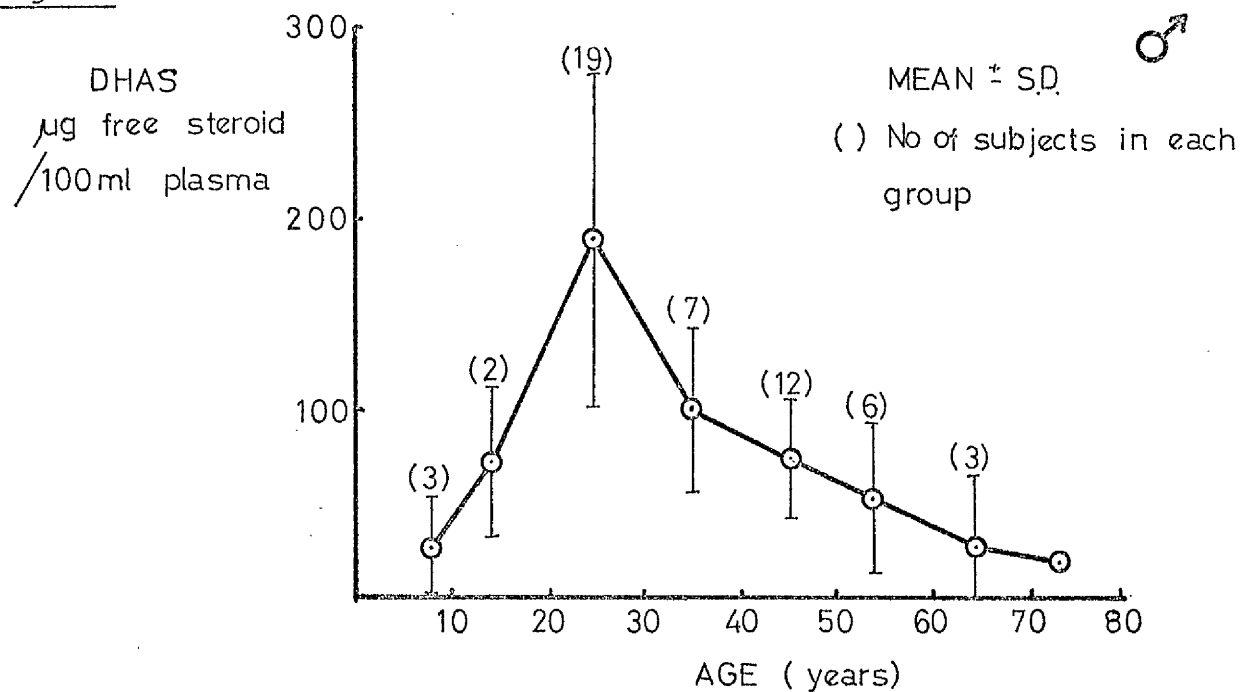
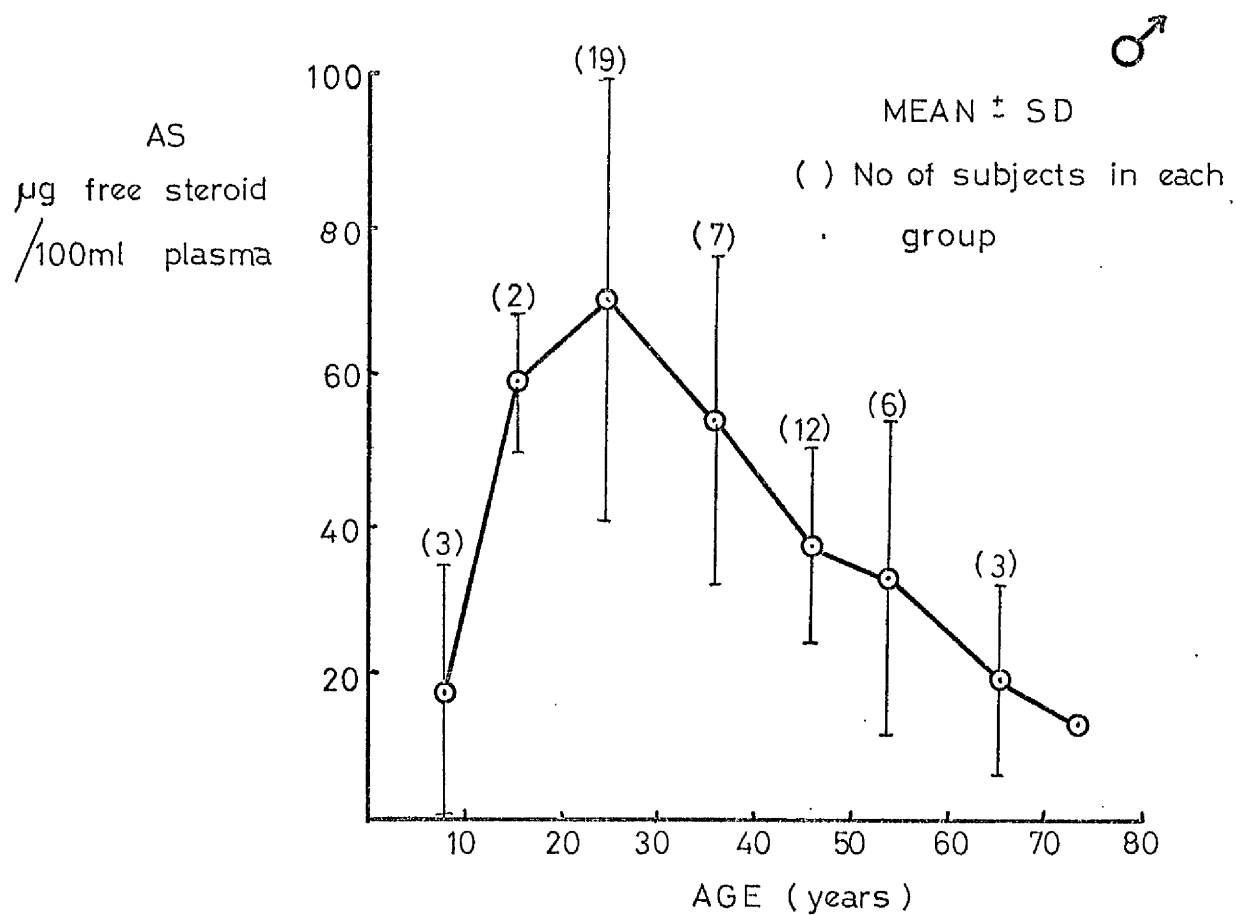


Fig. 10b



Plasma levels of individual 17-oxosteroid sulphates

Fig 11a

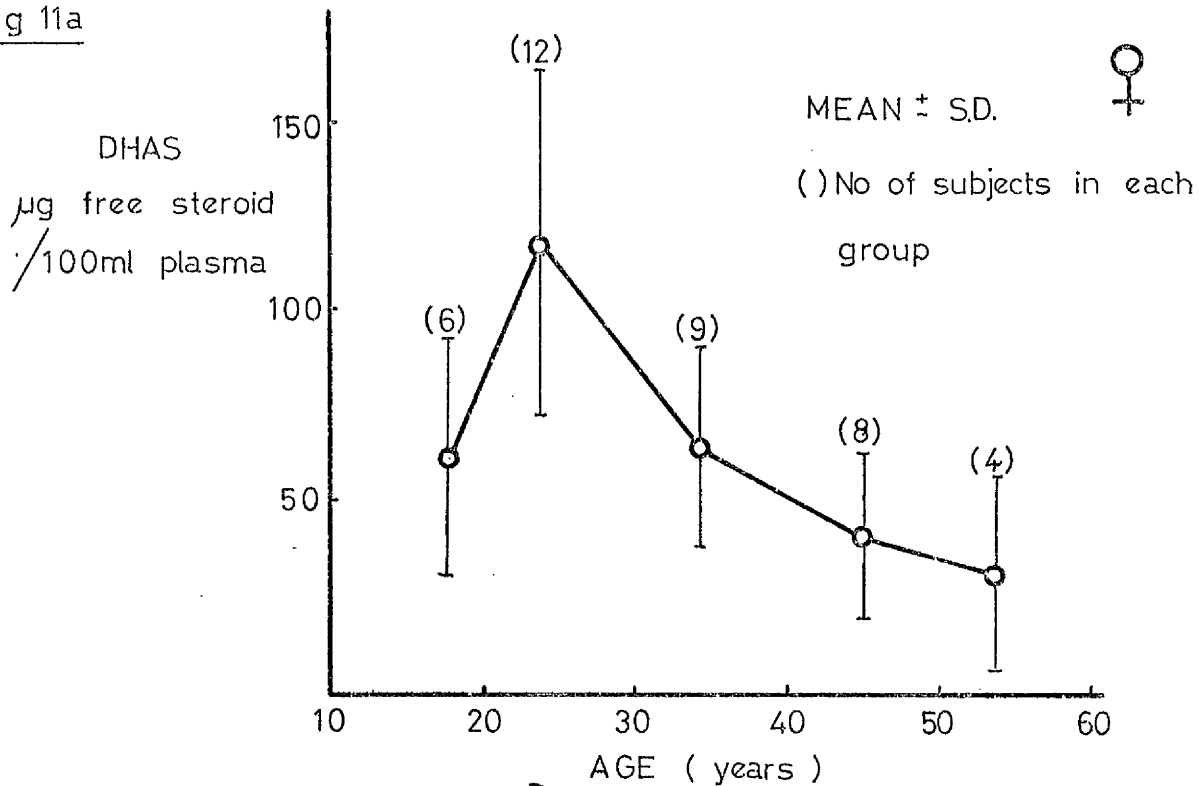
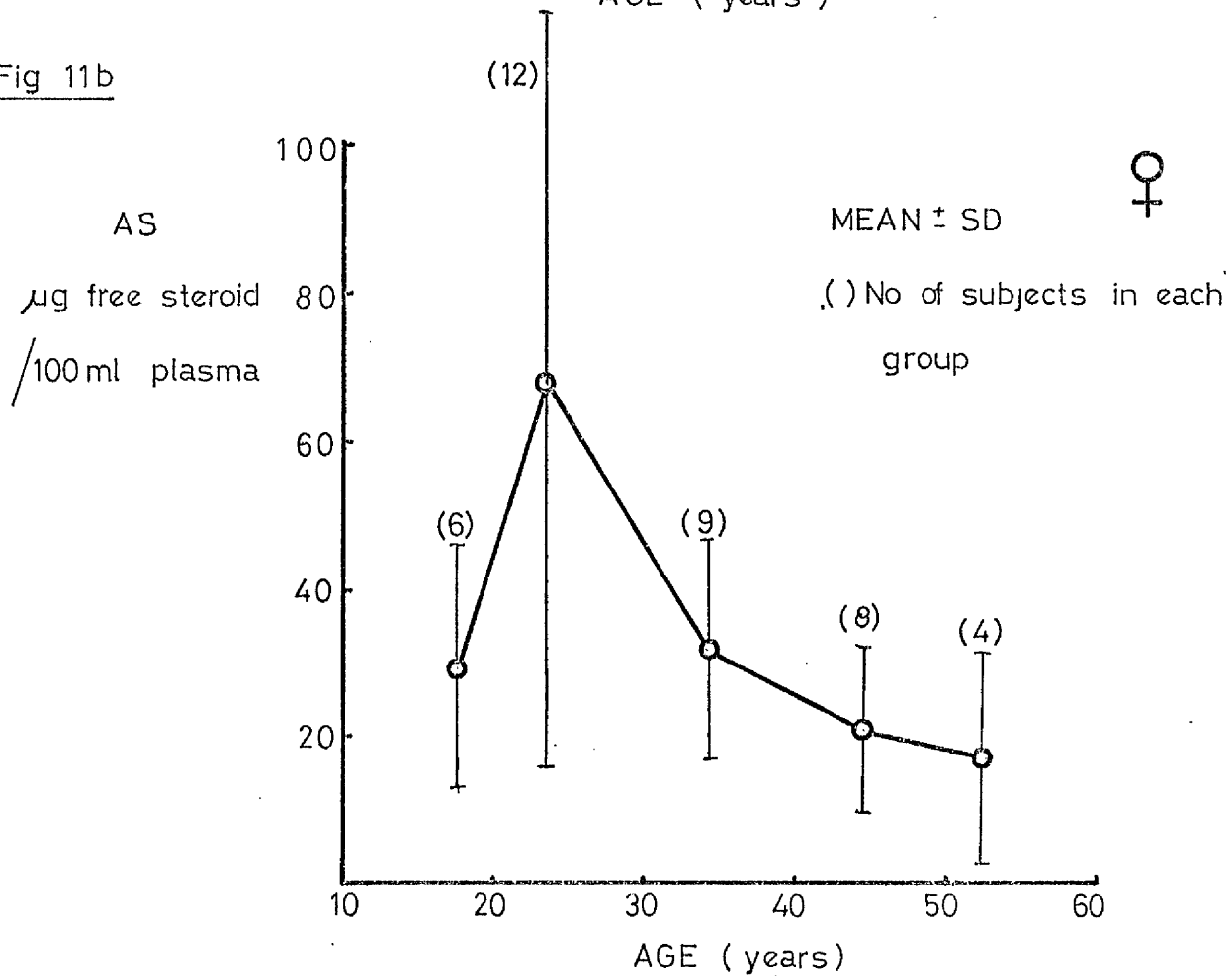


Fig 11b



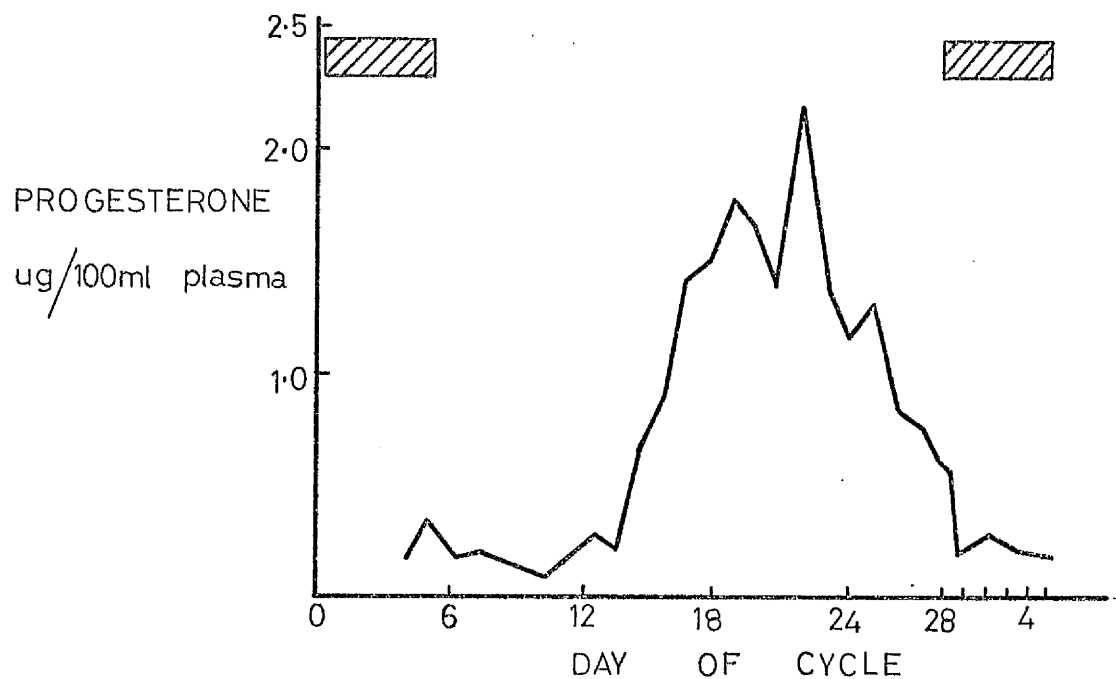
(From this Thesis)

of this is not clear. Levit et al. (1957) reported a young woman with exacerbations in the premenstrual phase of the cycle. With this case it was possible to precipitate attacks with progesterone, and Levit and his co-workers believed that this was due to increased progesterone in the luteal phase of the cycle. The magnitude of the increase of progesterone and its metabolites during the normal menstrual cycle is shown in Fig. 12. Oestrogen secretion, however, is also increased in the luteal phase (Fig. 13), and Redeker (1963) has precipitated acute attacks by the administration of a variety of exogenous oestrogens.

There is some disagreement whether onset or provocation of porphyria occurs in pregnancy. Certainly, there is a rise in the production and excretion of oestrogens and progesterones as pregnancy proceeds (Fig. 14a and b), and in general most investigators feel that a detrimental relationship exists between these two conditions (Reidenberg, 1955; Nielson and Nielson, 1958; Petrie and Mooney, 1962; Rimington and De Matteis, 1965; Tweedie and Mattox, 1965; Rovinsky and Guttmacher, 1965). One report by Nielson and Nielson (1958) of forty patients mostly with AIP in pregnancy revealed an exacerbation of symptoms in 95% and an alarming maternal death of 42.5%, with 58% of these deaths in primigravidas. Another study of fifty-five pregnant patients with porphyria by Rovinsky and Guttmacher (1965) showed that more than 75% of pregnancies coincided with exacerbations of

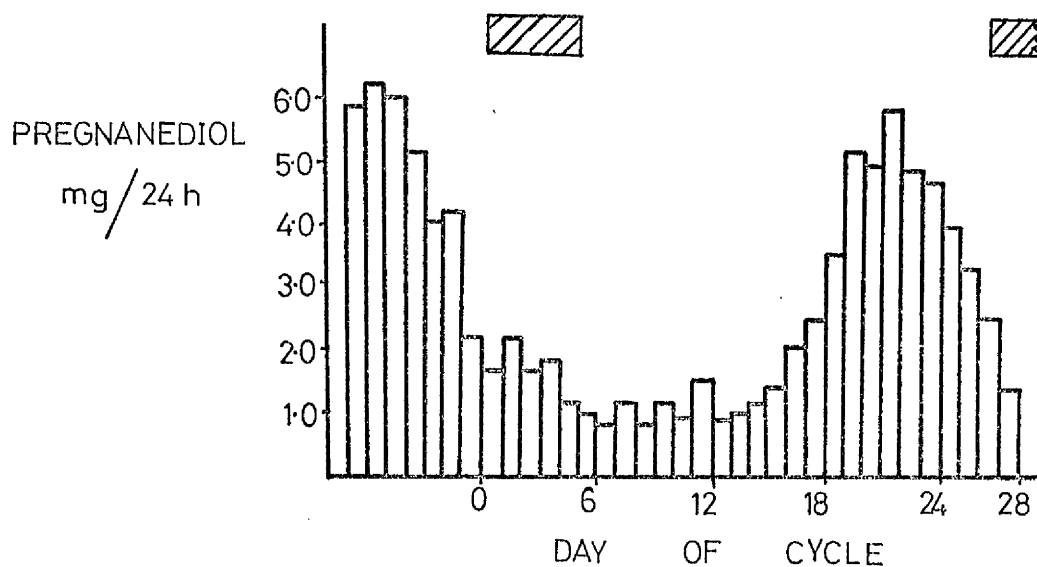
Levels of progesterone and a metabolite during a normal menstrual cycle

Fig. 12a



Plasma progesterone level in a normally menstruating subject. (From Woolever, 1963)

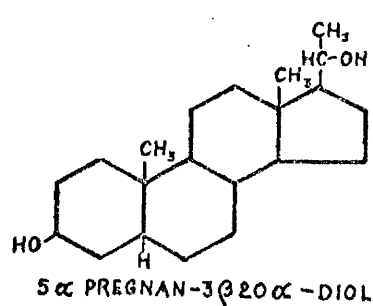
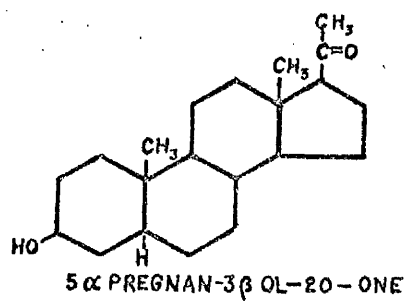
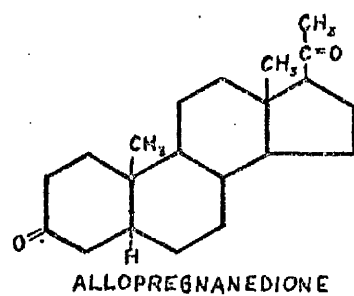
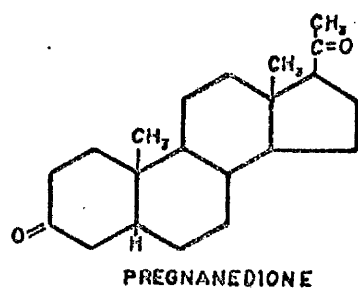
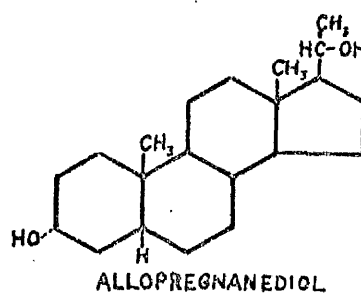
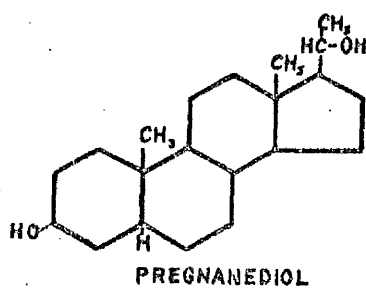
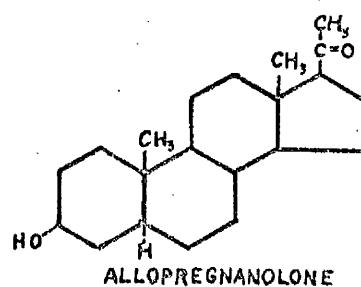
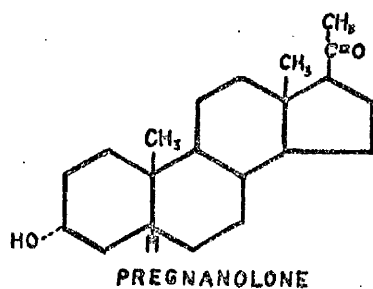
Fig. 12b



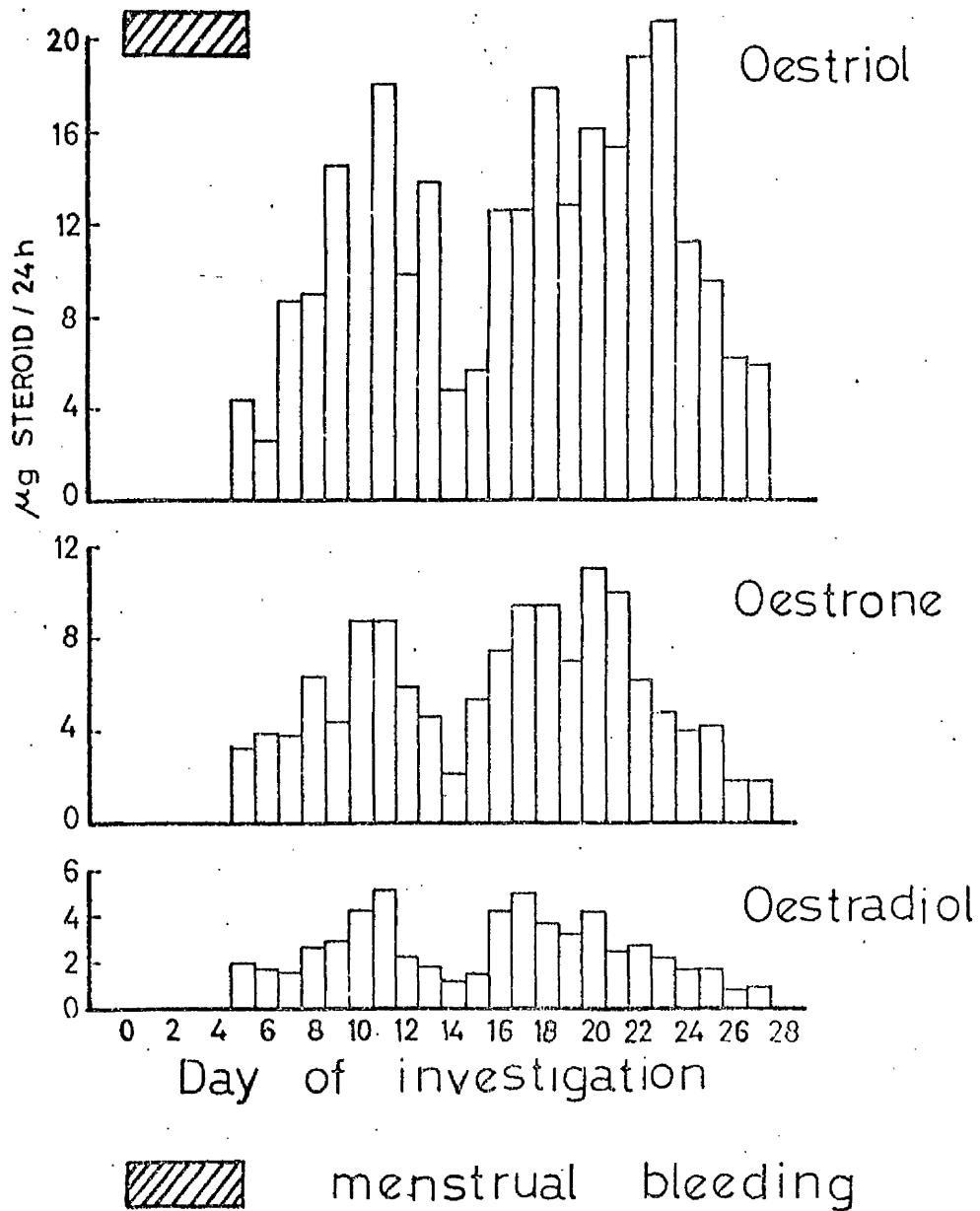
Urinary excretion of pregnanediol in a normal adult menstrual cycle.
(From Kloppe, 1957)

Figure 12c.

Urinary Progesterone Metabolites (C₂₁)



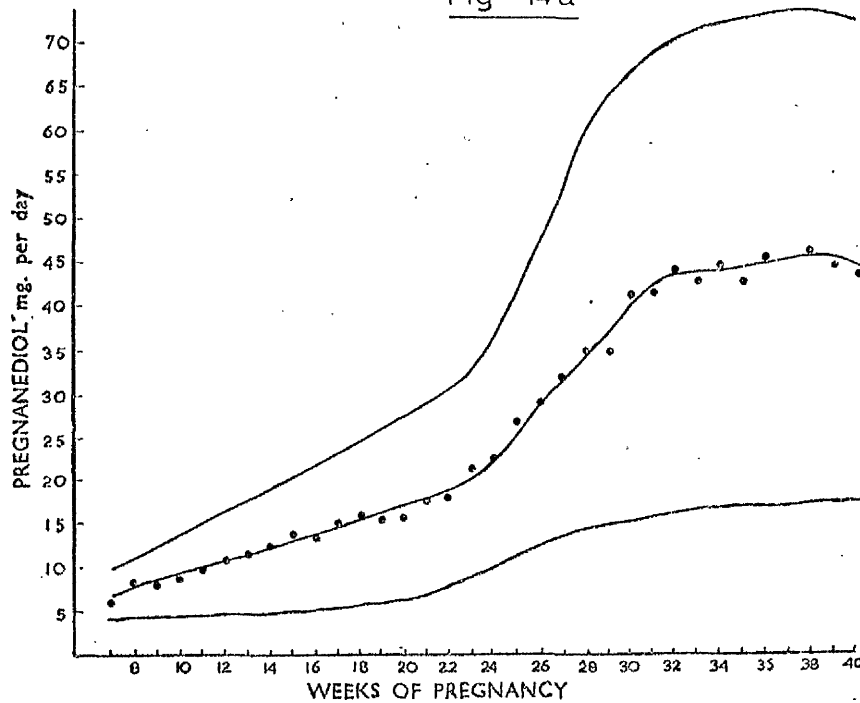
Urinary excretion of oestrogens during the normal menstrual cycle



(From Nocke and Breuer, 1963)

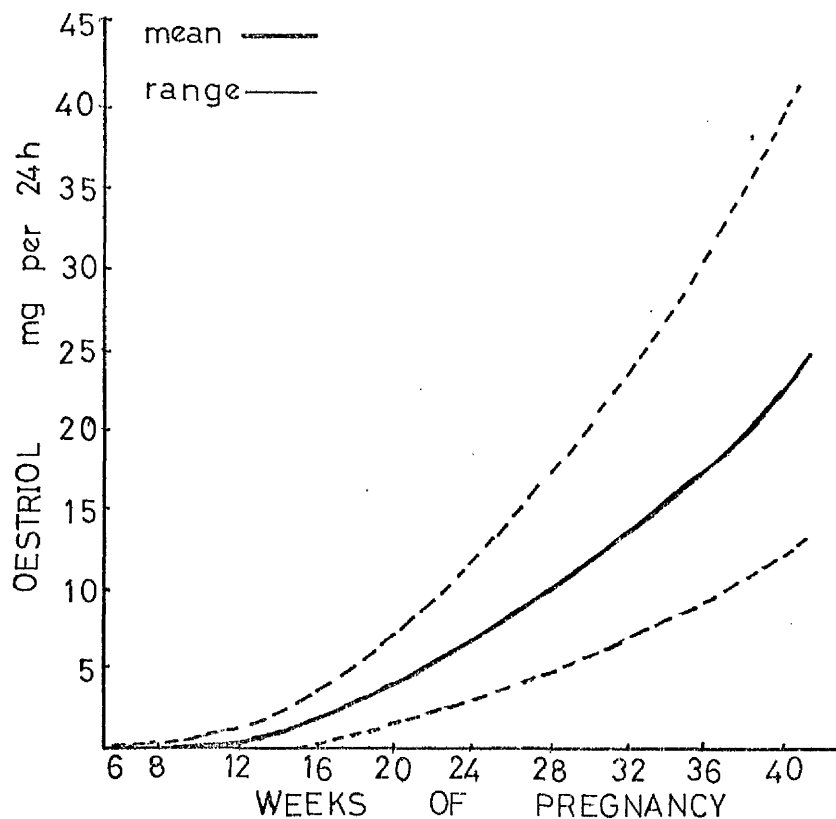
Steroid levels during pregnancy

Fig 14a



Pregnanediol excretion in normal pregnancy. The solid circles represent observed means, the central line fitted means. The upper and lower lines show the limits of 95per cent probability.
(from Shearman, 1958)

Fig. 14b



Urinary excretion of oestriol during thirty-six normal pregnancies.
(from Coyle and Brown, 1963)

symptoms. The overall maternal mortality rate cited in this publication was 22.4%, 15 of the 17 deaths occurring in primagravidas and 8 occurring with the first attack. Development of acute manifestations during pregnancy has also been described in patients with HCP by Goldberg et al. (1967), Connon and Turkington (1968) and Dean et al. (1969) and in patients with VP by Linas et al. (1947), Coleman (1948), Dean (1953) and Neilson and Neilson (1958). In contrast to this, Bloch (1965) concluded from his study of pregnancy in sufferers from porphyria variegata that the disease has no effect on pregnancy, nor does pregnancy adversely affect the disease. There have also been reports of patients suffering from acute intermittent porphyria showing remarkable clinical improvement on becoming pregnant (Rawlings, 1950; O'Dwyer, 1955). O'Dwyer was of the opinion that adrenal insufficiency was partly responsible for the clinical symptoms of porphyria and that pituitary hyper-activity in pregnancy compensated for this deficiency, resulting in a favourable outcome for his patient.

The relation of steroid hormone administration to hepatic porphyria appears to be a complex one, since they can either precipitate acute attacks or, in some patients in whom exacerbations of clinical symptoms occur regularly with menstruation, prevent the appearance of acute attacks.

Watson (1964), Eales (1963) and Dean (1965) have observed both exacerbations in skin lesions and haem

precursors in variegate porphyria following administration of oestrogen and/or progesterone compounds. Similarly, there has been a number of cases recorded in which a marked increase in porphyrin excretion and the appearance of clinical symptoms of photosensitivity have been observed in association with the administration of oestrogen and in which there was no obvious evidence of a genetic background (Hurley and English, 1963; Warin, 1963; Becker, 1965; Copeman et al., 1966; Felsher and Redeker, 1966; Levere, 1966; Zimmerman et al., 1966; Thivolet et al., 1967; Vail, 1967). The majority of these patients were given the hormonal preparation for prostate carcinoma, and an interval of several months or years had elapsed between the starting of the therapy and the appearance of the cutaneous symptoms. No evidence for a familial occurrence could be obtained from the histories of the patients, and biochemical analysis of the excreta from their relatives revealed normal porphyrin excretion in the few cases in which these examinations were carried out. Mild impairment of liver function was not uncommon among the patients (Zimmerman et al., 1966). Withdrawal of oestrogens led to a gradual improvement in the cutaneous symptoms (Zimmerman et al., 1966), although porphyrin excretion remained elevated in all six patients in which it was followed (Warin, 1963; Becker, 1965; Copeman et al., 1966; Zimmerman et al., 1966). Levere (1966) reported a considerable increase in the activity of liver ALA.S in

a case of cutaneous porphyria which followed the administration of oestrogens.

A number of observations have been recorded on the effect of oestrogens and progestogens (administered either separately or in combination, as frequently used in oral contraceptives) on the biochemical picture and clinical state of patients with AIP. Increased excretion of porphyrin precursors and also, less frequently, appearance of symptoms indicative of a clinical exacerbation have been observed in several patients of both sexes by Levit et al. (1957), Redeker (1963), Welland et al. (1964) and Wetterberg (1964). In complete contrast, in the patient described by Haeger-Aronsen (1963) and in the three cases reported by Perlroth et al. (1965), the exacerbation of AIP occurring in association with menstruation was prevented by the administration of synthetic sex hormones. In these four cases, different hormonal preparations, each containing an oestrogen together with a progestogen, were used.

This striking difference in response to the hormonal treatment in the two groups of patients and the periodic exacerbation of the disease with menstruation and pregnancy, which can be observed in some porphyric patients, emphasize the importance of the hormonal state in the pathogenesis of the attacks of the disease. It is not known in what way the hormonal state is implicated or which endocrine system is primarily involved. In one of the patients described by Perlroth et al. (1965), complete control of the porphyric

symptoms was obtained by treatment with either an androgen or an oestrogen alone, as well as by administration of a combination of an oestrogen with a progestogen. Androgenic therapy was similarly effective in another patient described by Schmid (1966). These observations suggest that a physiological response common to all three hormonal treatments may prevent the acute attacks in these patients. Perlroth et al. (1965) have suggested the inhibition of the secretion of the pituitary gonadotrophins, with stabilization of endogenous steroid production at a low level, may be the effective mechanism. This interpretation presupposes that a high level of endogenous steroids may be conducive to an attack of acute porphyria, whereas a high level of exogenous synthetic steroids may not. This concept is not supported by the finding by Redeker (1963) and Wetterberg (1964) of acute episodes after administration of synthetic steroids.

The inimical effect of oestrogens on cutaneous porphyria appears to be more consistent, and it seems probable that the cutaneous symptoms observed might be explained by the impairment of liver excretory function by prolonged administration of these oestrogenic compounds. It has been demonstrated by Mueller and Kappas (1964) that increased sulphobromophthalein (BSP) retention occurs in human subjects after parenteral administration of oestriol and oestradiol. Further studies by Kottra and Kappas (1967) have shown that this retention was not due to major hormonal impairment of conjugation processes for BSP. Combes and his associates (1963) have reported that

hepatic excretory activity is regularly depressed during pregnancy and that this functional defect can be attributed largely to the pharmacological consequences of the large amounts of oestradiol, oestriol and related compounds produced during gestation (Fig. 14). Eisalo et al. (1964) described impaired liver function after administration of oral contraceptives, apparently due to their oestrogenic compound. A significant elevation of urinary coproporphyrin I and ALA has also been found in pregnancy, especially when associated with hepatic disease (Koskelo and Toivonen, 1968) and in women on oral contraceptives (Koskelo et al., 1966). These findings suggest that impairment of liver excretory function may be partly responsible for increased urinary excretion of porphyrins and precursors, and for the appearance of symptoms of photosensitivity in symptomatic porphyria (PCT symptomatica). This opens the question of whether or not the mechanism of induction of porphyria by oestrogenic compounds may occur, in part at least, indirectly by establishing a condition of liver damage in these people with an inherited susceptibility for porphyria. An indirect mechanism of this type may perhaps explain the reason for the length of time taken by the majority of these oestrogenic agents to cause increased porphyrin excretion and symptoms of cutaneous porphyria (Walshe, 1963; Copeman et al., 1966; Levere, 1966; Degos et al., 1969; Roenigk, 1970), and also the reason for the persistence of the biochemical manifestations in spite of the withdrawal of the causative agent once the metabolic

disorder is established (Becker, 1965; Copeman et al., 1966; Zimmerman et al., 1966). Further support for this suggestion is provided by the observations that the clinical and biochemical evidence of liver damage is much more common in symptomatic porphyria than in the other varieties of hepatic porphyria, and by the reports that increased porphyrin excretion and appearance of symptoms of photosensitivity are observed in chronic liver disorders in which there is no history of alcoholism, or of prolonged exposure to any of the hormonal preparations, drugs or other toxic factors associated with hepatic porphyria (Brunsting, 1954; Tio et al., 1957; Braun and Berman, 1959). Shanley et al. (1969) have reported that conjugation of aetiocholanolone, a potent inducer of porphyrin formation in chick embryo liver cell culture (Granick and Kappas, 1967), was found to be impaired both in patients with symptomatic porphyria and in non-porphyric patients with liver disease compared to normal subjects. They suggest that faulty regulation of steroid conjugation might play a secondary role as an aggravating factor in symptomatic porphyria. Similarly, Zumoff et al. (1967 and 1966) have reported that steroid glucuronide formation in patients with liver cirrhosis was reduced between 41% and 87% of normal.

The induction of ALA.S in chick embryo liver culture by some of these sex hormone preparations has been investigated by Rifkind et al. (1970). They reported that the active inducer component of these contraceptive steroids was the

progestogen, whilst no oestrogen significantly altered the level of the enzyme on its own. In contrast to this, oestradiol, a naturally secreted oestrogen, has been shown by Tschudy et al. (1967) to produce a series of oscillations of the level of hepatic ALA.S in ovariectomized rats. These oscillations continued for as long as 90 hours, and Tschudy and his associates suggested that they resulted from a perturbation of the closed negative feedback loop which controls the synthesis of hepatic ALA.S.

Granick (1966) was the first to report that several steroids of physiological origin in man have significant capacity for induction of porphyrins in primary culture of chick embryo liver cells. In subsequent publications, Granick and Kappas (1967a,b) and Kappas and Granick (1968) described more exhaustively the effects of a series of steroids on porphyrin metabolism. They found that the most potent inducers of porphyrin synthesis in chick embryo liver culture were the C₁₉ and C₂₁ 5 β -H neutral steroids (Fig. 15a,b). These certain steroids all share the basic nuclear structure of 5 β -H (A : B cis) compounds in which the junction of the A and B rings is highly angular rather than planar, and they are all derived from the in vivo biotransformation of steroid hormones or intermediates rather than being primary endocrine secretions themselves (Fig. 16). Several of them, such as aetiocholanolone, pregnanediol and pregnanolone, are produced in significant amounts daily from the metabolic conversions of such precursor hormones as testosterone and progesterone.

Figure 15a

STEROID STRUCTURE

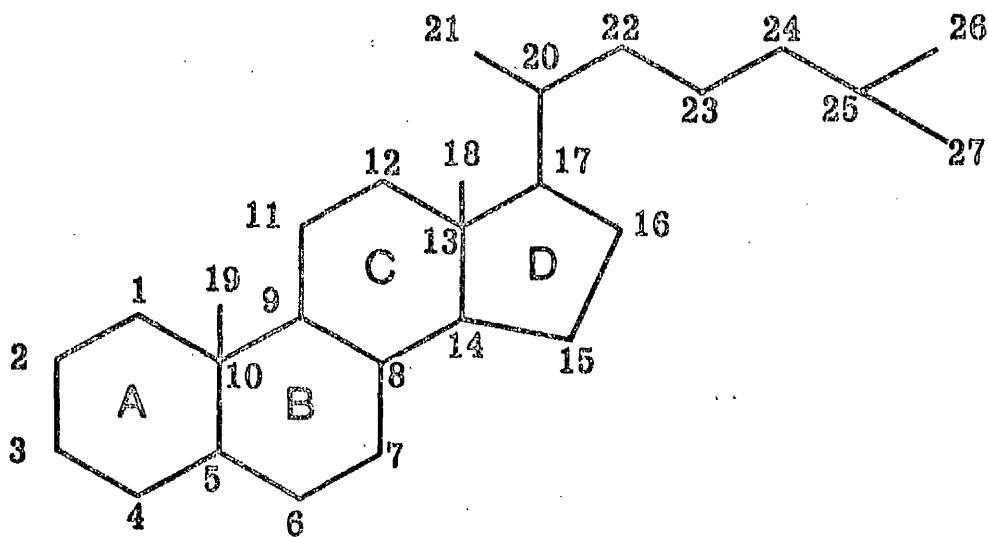
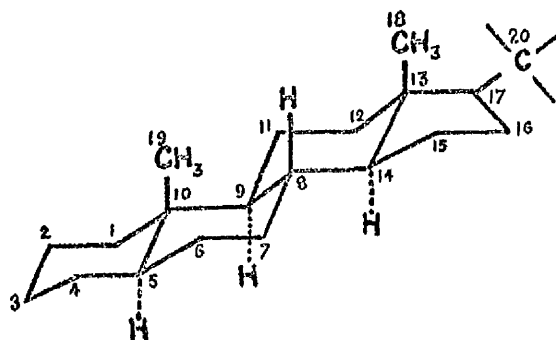


Fig. 15a.1 The numbering system of the steroid nucleus and the nomenclature of its rings.

5 α -Series



5 β -Series

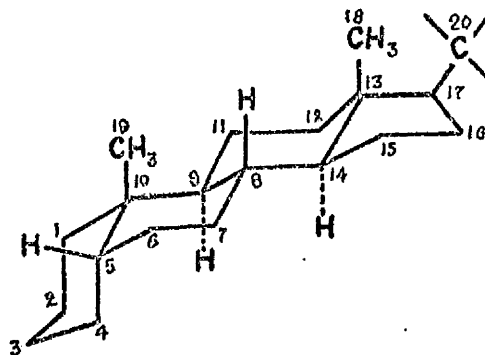


Fig 15a.11 Stereochemical Representations of the basic nuclear structures characteristic of 5 α -H (A : B trans) and 5 β -H (A : B cis) steroid metabolites. The differences in the conjugation of the A : B-ring junction in these two series of metabolites is evident.

STEROID FORMULAE

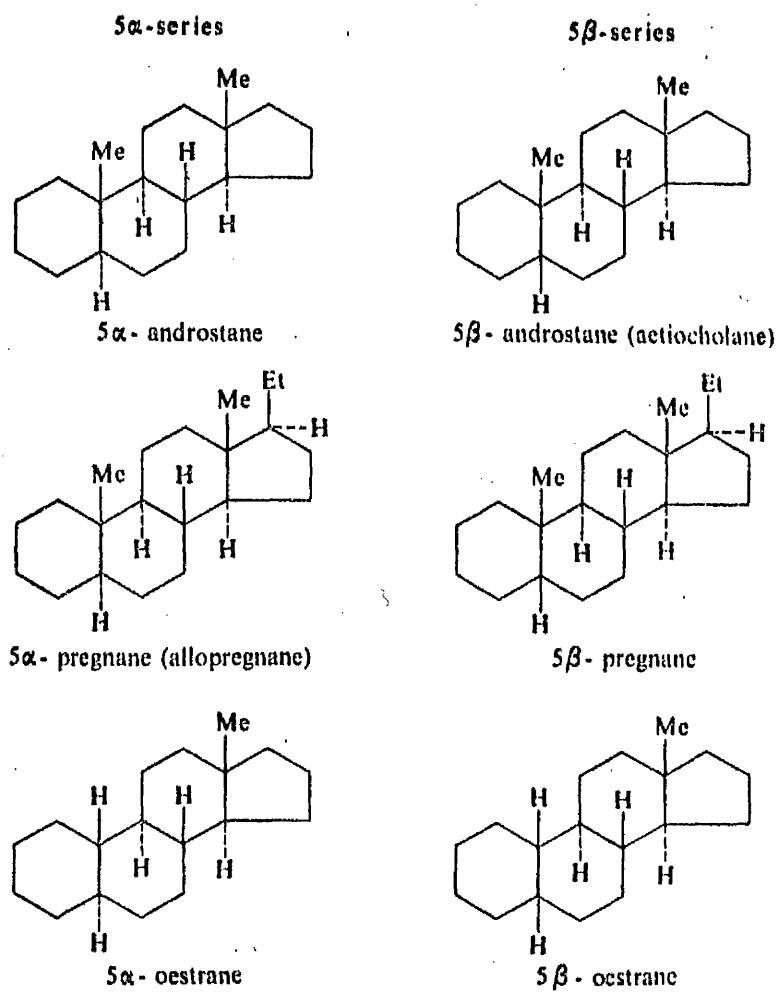
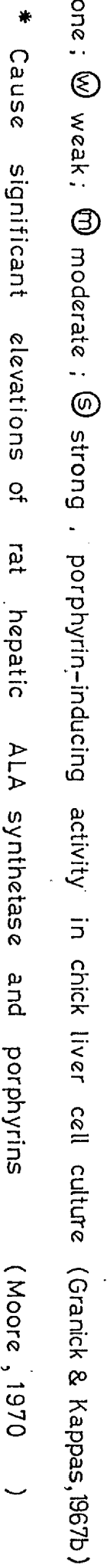


Fig. 15b The names and formulae of the C₁₉, C₂₁ and C₁₈ steroid parent compounds (Me - Methyl; Et - Ethyl)

STEROID METABOLISM



* Cause significant elevations of rat hepatic ALA synthetase and porphyrins (Moore, 1970)

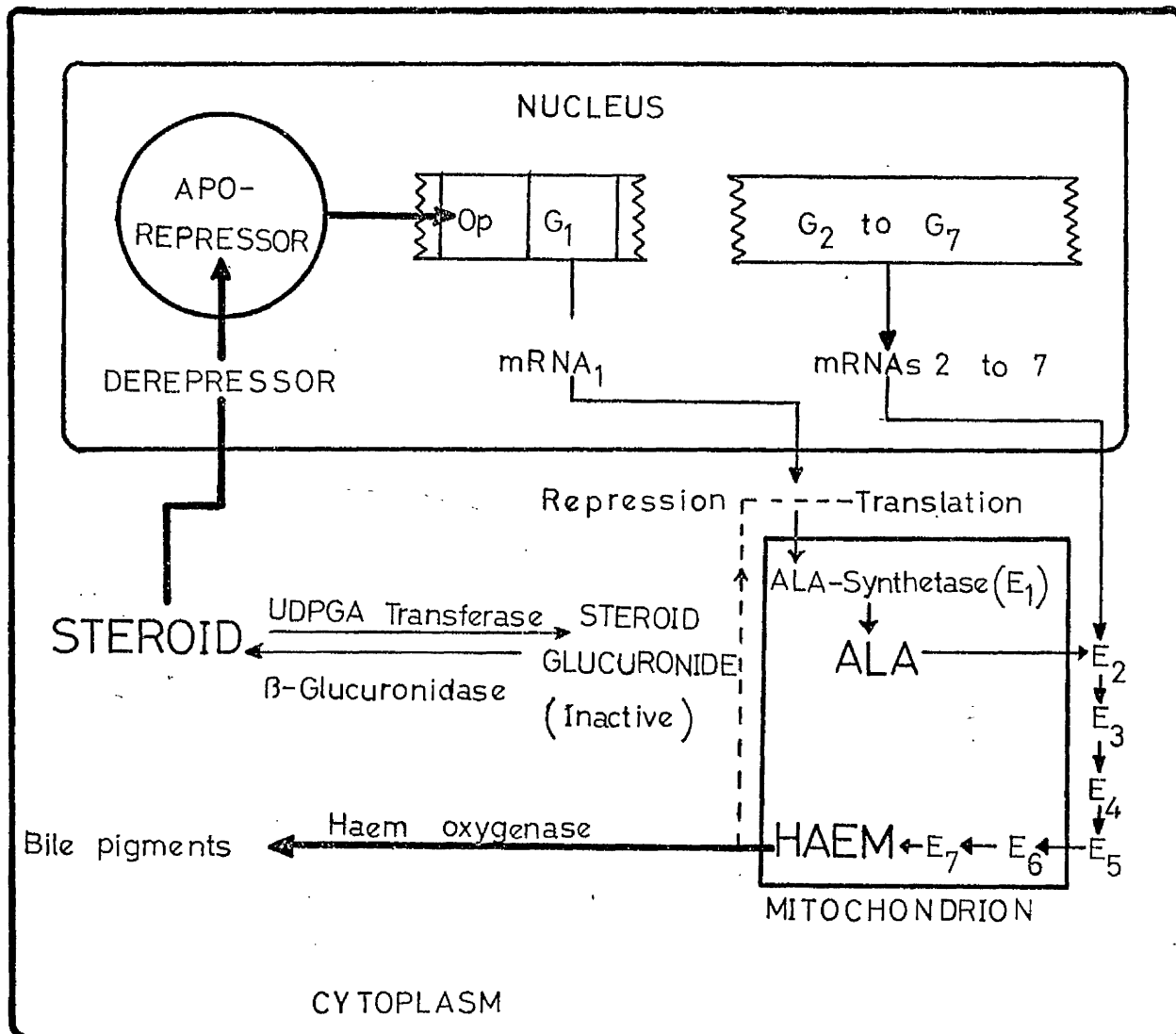
They further showed that in the conjugated glucuronide form these steroids did not induce porphyrin synthesis in culture, and also that this induction of porphyrin synthesis by these free steroids was abolished by uridine diphosphoglucuronic acid (UDPGA) and by inhibitors of nucleic acid and protein biosynthesis, like actinomycin D and puromycin. This prevention of the inducing action of free steroids by the concomitant addition to the culture of UDPGA is considered to reflect the enhanced conversion in the culture of the active free steroid to the inactive glucuronide conjugate, since this nucleotide (UDPGA) is the immediate donor of glucuronic acid in conjugation reactions catalysed by UDP glucuronyl transferase (Dutton, 1966). Granick and Kappas (1967a) further suggested that any process which led to - a) impaired glucuronidation of the steroid due to inhibited or defective UDP-glucuronyl transferase, or limited UDPGA formation; b) enhanced intracellular hydrolysis of formed conjugates by excessive β -glucuronidase activity; c) steroid production in amounts exceeding the body's capacity to dispose of them; - would result in induction of ALA.S and increased production of ALA and other precursors of haem. The diminishing of the inducing effect of drugs and steroids on porphyrin formation by a high glucose administration (Rose et al., 1961; De Matteis, 1964) is thought to be partially due to an increase in the products of the glucose phosphate-pentose-P pathway, especially UDP-glucuronic acid which may serve for glucuronidation and thus solubilize the drug or steroid so that it is

readily removed by excretion (Sassa and Granick, 1970). Similar effects of fasting and high carbohydrate intake have been observed in patients with AIP by Welland et al. (1964). It is of interest that 5 β -H steroids like pregnenolone may have an inhibitory activity on glucuronyl transferase (Lathe and Walker, 1958), so that theoretically the increase in such a steroid which induces ALA-S may tend to block glucuronidation by setting up a self-sustained cycle of progressively increasing steroid concentration, resulting in clinical relapse of acute porphyria (Kappas and Granick, 1968).

The synthesis of the individual porphyrins in chick embryo liver cells growing in culture following induction by meprobamate and aetiocholanolone has been investigated by Doss and Kaltefleiter (1971). They showed that with either of these two inducing substances, protoporphyrin (proto), the end product of the porphyrin chain was the first to accumulate and, after stimulation of porphyrin synthesis, proto predominated over the higher carboxylated porphyrins. Similar relatively high values of proto in the livers of animals with experimentally induced porphyria have been reported by various investigators (Schmid et al., 1955b; Granick and Urata, 1963; Onisawa and Labbé, 1963; Janousek, 1970). These findings serve to demonstrate that chemically induced porphyrin synthesis in liver cells in vivo and in vitro shows striking similarities.

As a result of their investigations, Granick and Kappas (1967a) and Kappas and Granick (1968) suggested that certain steroids of physiological origin in man induce the 'de novo' synthesis of ALA.S in chick embryo liver cell culture, and a hypothesis involving the repressor-inducer mechanism for gene activation proposed by Jacob and Monod (1961) was presented for the control mechanisms of synthesis; one at the transcriptional and the other at the translational level (Sassa and Granick, 1970). They showed that aetiocholanolone (5 β -H oxosteroid) and DDC (3,5-diethoxycarbonyl-1,4-dihydrocollidine) induce at the transcriptional level, whereas AIA (allyl-isopropylacetamide) and Lindane (a non-planar insecticide, hexachlorohexahydrobenzene) induce primarily at the translational level. In this publication they also reported that haem inhibits at the translational rather than at the transcriptional level, as had been previously hypothesized (Granick, 1966). The results obtained by Sassa and Granick (1970) suggest that haem inhibits either by shortening the lifetime of the mRNA for ALA.S or by interfering specifically with the synthesis of ALA.synthetase. As a result of their investigations, Granick and Sassa (1971) suggested that the dominant Mendelian inheritance of AIP is most simply explained as a defect in an operator gene that controls the operon for the synthesis of mRNA for ALA.S (Fig. 17). On the basis of this assumption, they proposed an operator-repressor mechanism at the transcriptional level, with inducers like

Figure 17.



Hypothesis on the controls of haem synthesis in the hepatic cell at both the transcriptional and translational levels. In the nucleus a repressor protein blocks transcription at the promoter end of the operator gene, thus preventing synthesis of the mRNA of ALA-synthetase. A 5β-H steroid removes the repression by interacting with the repressor protein. The level of the steroid is controlled by glucuronidation with UDPGA transferase which renders it inactive, and by hydrolysis with β-glucuronidase which renders it active. In the cytoplasm haem represses the synthesis of ALA-synthetase at the translational level and thus its own synthesis. Excess haem is destroyed by haem oxygenase of the microsomes.

5 β -H steroids or chemicals like DDC reacting with the repressor to derepress or unblock the operator. The free operator would then allow transcription of the structural gene to take place, resulting in mRNA for ALA.S. At the translational level, they proposed a haemoprotein as a repressor with haem serving as a co-repressor. The action of this haemoprotein would prevent the mRNA of ALA.S from being activated or translated. Inducers like AIA or Lindane would act indirectly to derepress the haemoprotein, perhaps by causing the destruction of the haem. Thus, this 'silent' mRNA, conceived as a complex of the repressor haemoprotein with the specific mRNA, might be converted to active mRNA. In addition to this hypothesis, Kurashima et al. (1970) have suggested that haemin might inhibit the activity of ALA.S by interfering with its transport into the mitochondria. Additional evidence of some control mechanism operating at the transcriptional level in the liver cell is presented by Incefy and Kappas's (1971) study of the effect of α -Amanitin, a highly specific inhibitor of mammalian RNA polymerase, on the induction of ALA.S by aetiocholanolone in chick embryo liver.

It was found by Levere et al. (1967) that the same 5 β -H oxosteroids that actively stimulate the synthesis of ALA.S in chick embryo liver cells, also stimulated haemoglobin synthesis in chick embryo blastoderm cells. These investigators inferred that this stimulation of haemoglobin synthesis by the steroids in the blastoderm cells is brought

about by the same mechanism as that for the liver; namely, induction of the synthesis of ALAS at the transcriptional level. Gorschein and Gardner (1970) observed that in mice made polycythemic and then treated with steroids and finally with labelled iron, there was increased label in the peripheral circulation. The controls contained 1% of the labelled iron in haemoglobin, whereas those treated with 5 β -pregnane-11,20-dione contained up to 15% of the labelled iron. No stimulation of this uptake of labelled iron was observed in those mice treated with 5 α -H steroids. Gordon et al. (1970) carried these investigations further. They noted that testosterone increased erythropoiesis by stimulating erythropoietin formation. They also showed, by preventing the induction of erythropoiesis by erythropoietin with antibody for this hormone, that the 5 β -H oxosteroids (11-ketopregnenolone, aetiocholanolone) stimulated erythropoiesis independently of erythropoietin. Studies of the stimulating effect of these 5 β -H oxosteroid metabolites on haem formation in human bone marrow cells were reported by Necheles and Rai (1969). They observed that aetiocholanolone (5 β -androstane-3 α -ol,17-one), but not androstanolone (5 α -androstane-17 β -ol,3-one) was effective in stimulating the incorporation of labelled glycine into haem. Their finding that this 5 β -H oxosteroid-induced stimulation of haem synthesis is completely abolished by both actinomycin D and puromycin suggests that the stimulating effect is mediated by newly formed RNA-dependent protein synthesis.

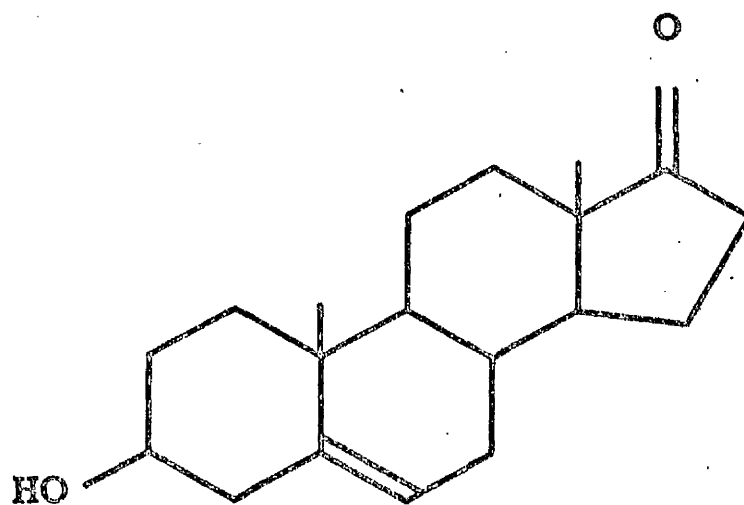
All these observations indicate that 5 β -H oxosteroids of

the androstane, pregnane and perhaps longer carbon skeleton series are active in the control of haem synthesis in both the hepatic and erythroid cells and could possibly act as persistent stimulants to porphyrin and haem production. This action could presumably intensify at times; for example, in the luteal phase of the menstrual cycle and pregnancy, in which, in certain patients, steroid production is increased beyond the capacity of the liver to metabolize, conjugate and excrete such substances. If this is the case, it would appear likely that these 5β -H oxosteroids play a significant role in susceptible persons in determining the time of onset of the disease; for example, at puberty, when there is a great rise in the production of steroids (Fig. 9).

These proposals indicate that steroid imbalance of some type might be associated with the porphyrias. Tschudy (1968) investigated the urine of several patients with hereditary hepatic porphyria but could find no evidence of elevated total urinary 17-oxosteroids. In the following year, Goldberg *et al.* (1969) reported elevated fractions of some urinary 17-oxosteroids in patients with AIP, although the total urinary 17-oxosteroids were within the normal range. In this investigation, the most striking steroid abnormality detected was the increased excretion of dehydroepiandrosterone (Fig. 18) (DHA), and it was shown that on injecting DHA or its sulphate conjugate into rats, a striking rise of hepatic ALA.S was obtained. In further studies in this system by Moore (1970), 17α -hydroxypregnenolone, androstenedione and aetiocholanolone

Figure 18.

THE STRUCTURE OF DHA



DEHYDRO-EPIANDROSTERONE (DHA)

(5-Androstene-3 β -ol-17-One)

were shown to have porphyrinogenic activity in the rat liver. In that same year, Kappas et al. (1969) demonstrated that the plasma from patients in attack of AIP was capable of strongly inducing the synthesis of porphyrins in chick embryo liver culture, and again nucleic acid and protein biosynthesis inhibition abolish this effect. The plasmas which contained the most potent porphyrin-inducing activities were derived from several patients with AIP during acute relapses of their disease; whereas, the plasmas of patients with hepatic porphyria in remission did not display significant inducing activity. It was also shown that a similar induction effect was produced by the plasma of some normal subjects receiving contraceptive steroid mixtures. Further evidence that some type of steroid imbalance exists in certain hepatic porphyrias was given by Gillette et al. (1970). They reported consistent increases of the 5β -H steroid metabolites as compared with the 5α -H steroid metabolites (which are normally excreted in equimolar amounts) in the urine of several patients with AIP. They suggested that this might be due to a deficiency of a Δ^4 - 5α -reductase enzyme activity.

There is also evidence that the glucocorticoids may be secondarily involved in the induction of porphyrin synthesis. Marver et al. (1966c) found that adrenalectomized mice did not become porphyric when treated with both AIA and hydrocortisone. Although the glucocorticoids cannot themselves induce, they may be important to bring about the metabolic conditions, like stimulation of gluconeogenesis during fasting, which permit the

inducing steroids or chemicals to induce. Matsuoka et al. (1968) observed a doubling of ALA.S activity in rat liver when, simultaneously with AIA, either hydrocortisone or triiodothyronine was administered.

These clinical and experimental observations mentioned in this and the previous sections present a substantial amount of evidence for the occurrence of a deleterious interplay of endocrine and genetic factors in the natural history of these hereditary porphyrias, especially those of the hepatic group. The porphyrin-inducing 5β -H oxosteroids that have been identified, delineate one category of endogenous agents through which such an endocrine-genetic interplay may be effected in some subjects, and it is the purpose of this thesis to examine the relevance of the part played by some of these oxosteroids in the pathogenesis of the hepatic types of porphyria.

SECTION IV

THE URINARY 17^{ox} STEROIDS

A. INTRODUCTION.

The 17-oxosteroids comprise of a group of steroidal compounds which have in common a ketone group at the C-17 position of the steroid nucleus. Some have androgenic properties (androsterone and dehydroepiandrosterone), whereas others, such as aetiocholanolone, are not known to be biologically active. All are derivatives of two parent hydrocarbons, 5 α -androstane and 5 β -androstane (aetiocholane), which differ in the spatial configuration of the hydrogen atom at the C-5 position and in the fusion of rings A and B in the steroid molecule (Fig. 19). Eight of the more important urinary 17-oxosteroids are shown in Figure 20. Four of these have an oxygen substituent at the C-11 position in the form of either an oxo (=O) or an hydroxyl (-OH) group. Isomerism at C-3 position is encountered relatively frequently in the 17-oxosteroids. This is well illustrated by comparing the structure of the two compounds, androsterone and epiandrosterone. When a group such as 3-OH is attached to the steroid nucleus above the plane of the molecule, i.e. on the same side as the angular methyl group at C-10 and C-13, such a linkage is termed β and is depicted as being connected to the rest of the molecule by a solid line. When the -OH group is below the plane of the molecule, this is designated α and the linkage is shown by a broken line. Epiandrosterone and DHA are the principal 17-oxosteroids with the 3 β -hydroxy group, whereas androsterone and aetiocholanolone are the two most important compounds

LEGEND FOR FIGURE 19

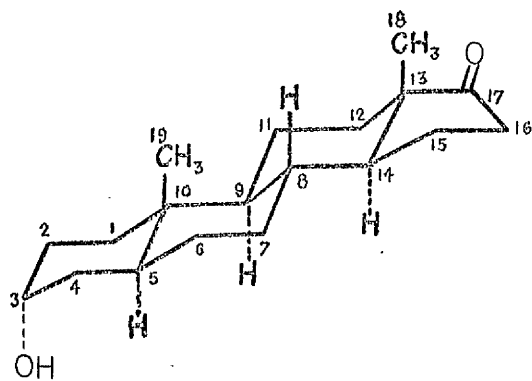
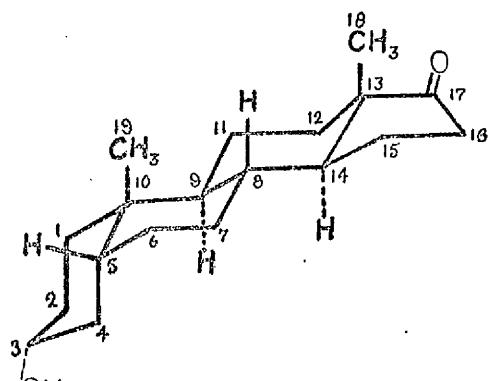
1. Androsterone and aetiocholanolone differ in the spatial configuration of the hydrogen atom at the C-5 position and in the fusion of rings A and B as shown by the top two steroid molecules on the opposite page.

2. Androsterone and epiandrosterone differ only in the spatial configuration of the hydroxyl group at the C-3 position.

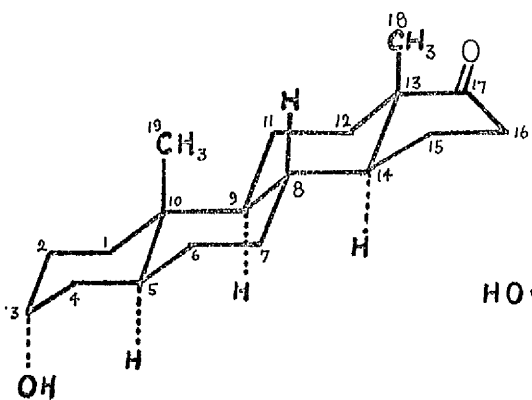
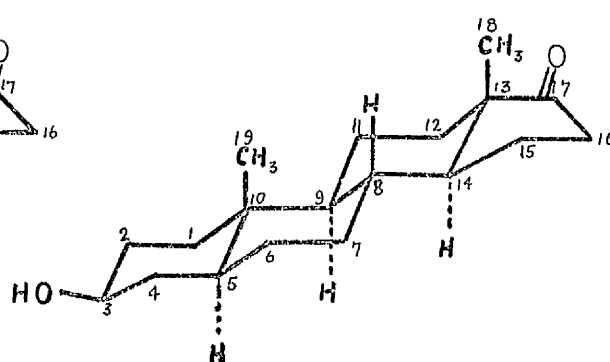
3. Dehydroepiandrosterone is unsaturated between the C-5 and C-6 positions, and this results in a bending of the steroid molecule between rings A and B.

Steroid structures

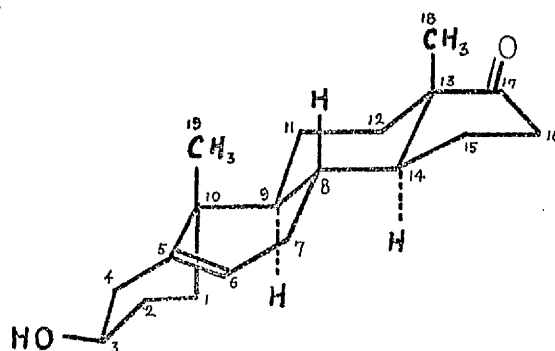
1.

ANDROSTERONE (5 α -H)AETIOCHOLANOLONE (5 β -H)

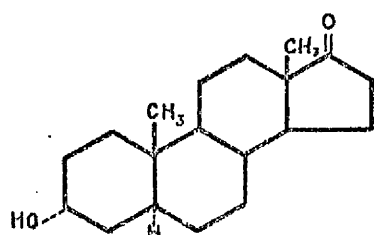
2.

ANDROSTERONE (3 α -OH)EPIANDROSTERONE (3 β -OH)

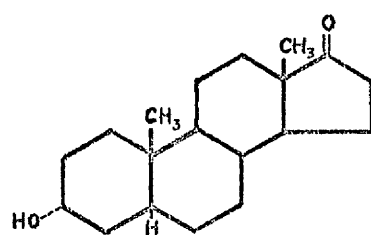
3.

DEHYDROEPIANDROSTERONE (unsaturated , Δ⁵)

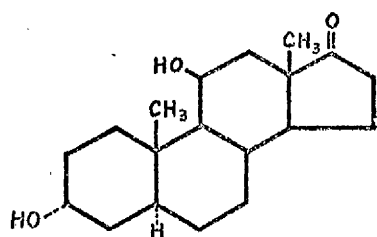
The principal urinary 17-oxosteroids



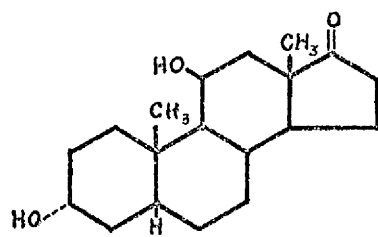
Androsterone



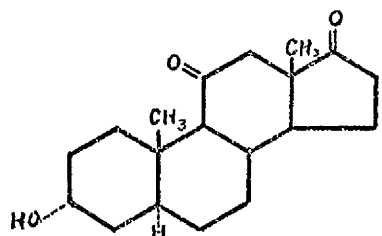
Aetiocholanolone



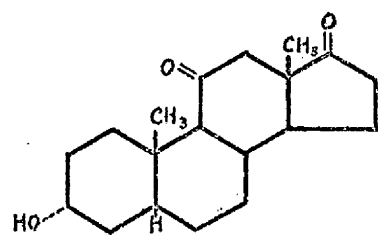
11β-Hydroxyandrosterone



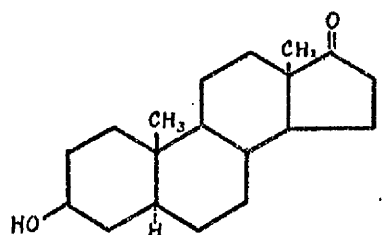
11β-Hydroxyaetiocholanolone



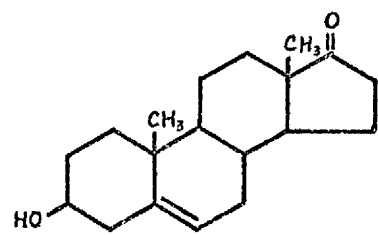
11-Oxoandrosterone



11-Oxo aetiocholanolone



Epiandrosterone



Dehydroepiandrosterone

with the 3α -hydroxy structure. It should be noted that the oestrogenic steroid, oestrone, is also a 17-oxosteroid. However, this compound, due to its phenolic structure in ring A, is acidic in nature and is removed from urinary extracts when these are washed with alkali. Accordingly, oestrone is not included in the terms 'neutral' 17-oxosteroids, which are the compounds under examination in this thesis.

The 17-oxosteroids are excreted in conjugation with glucuronic and sulphuric acids, rather than in the free form in the urine. It is generally believed that the urinary 17-oxosteroids originate from precursors such as DHA, DHAS, Δ^4 -androstenedione and testosterone, which have been excreted by the adrenal cortex, by the testes and possibly to a small extent by the ovaries (Van de Wiele *et al.*, 1963; Baulieu *et al.*, 1965; Dorfman and Sharma, 1965; Mikhael, 1970). Both DHA and testosterone are converted to androstenedione, which is converted into equal portions of androsterone (A) and aetiocholanolone (E) by Δ^4 -reductase (5α and 5β) of the liver (Baulieu and Mauvais-Jarvis, 1964a,b). These enzymes are susceptible to various precursors and hormonal influences and the E:A ratio may be shifted as a result of these influences. Exogenous DHA suppresses Δ^4 - 5α -reductase, and a rise in aetiocholanolone (E) develops, much as in malignancy of adrenals with excess DHA formation (Kirschner and Lipsett, 1964). Triiodothyronine stimulates Δ^4 - 5α -reductase, resulting in an increase of androsterone.

Deoxycortisol and other corticoids are metabolized to aetiocholanolone (MacDonald et al., 1962; Wilson and Schenker, 1964). In males, approximately one-third of the total urinary 17-oxosteroids are thought to represent metabolites of the testicular hormone, testosterone, while the remaining two-thirds are derived from steroids which have been elaborated by the adrenal cortex. In females, who usually excrete smaller quantities than males, the main site of production of the precursors of the urinary 17-oxosteroids is the adrenal cortex (Loraine and Bell, 1971).

The urinary 17-oxosteroids which are of adrenal origin arise in two different ways, being formed partially by oxidative removal in the tissues of the side-chain of C-21 steroids and partially from C-19 steroids which have been secreted by the adrenal gland itself (Loraine and Bell, 1971). The four main 17-oxosteroids secreted by the adrenal cortex are 11 β -hydroxyandrostenedione (Griffiths et al., 1963), androstenedione and DHA (Cohn and Mulrow, 1963), DHAS (Baulieu, 1962; Wieland et al., 1963) and androsterone (Bush et al., 1956; Short, 1960). Current views suggest that the principal C-19 steroid synthesized by the human adrenal cortex is DHAS (Wieland et al., 1963; Baulieu et al., 1965). In addition to the adrenals, DHAS can be formed from free DHA and other steroid precursors by the liver (Schneider and Lewbart, 1956) and by the intestinal wall (Baulieu, 1963b); DHA can also be sulphoconjugated by

the foetus (Wengle, 1964). The results obtained by Chapdelaine et al. (1965) also indicate that free DHA but not DHAS is secreted by the testes.

DHAS is far from being an end product; it undergoes metabolism either directly without splitting of the ester link to other steroid sulphates or indirectly by other catabolic pathways initiated by hydrolysis, leading essentially to the formation of androsterone and aetiocholanolone in the liver, which are then conjugated either with sulphuric or glucuronic acid and eventually excreted in the urine (Baulieu et al., 1965). The total urinary excretion of the conjugates of androsterone and aetiocholanolone includes a contribution from a testicular origin. This mainly arises from the metabolism of testosterone in the liver by two major pathways, both leading to the production and excretion of the conjugates of the neutral 17-oxosteroids (Van de Wiele et al., 1963; Dorfman, 1969). In women, a very small amount of testosterone is secreted both by the ovaries and the adrenals (Ismail and Harkness, 1966; Horton, 1966; Horton et al., 1966). In addition, the ovaries secrete relatively large quantities of the C-19 steroid, androstenedione, which is believed to be an important precursor of testosterone (Horton et al., 1966).

The major sources of the urinary 11-oxygenated 17-OS are the adrenal production of 11 β -hydroxyandrost-4-ene-3,17-dione and the peripheral metabolism of hydrocortisone and

possibly certain of its reduction products (Goldzieher and Beering, 1969). The results of their investigations indicate that within this sub-group of 11-oxy 17-OS, compounds with a 5β configuration are likely to be metabolites of the corticosteroids, whereas 5α compounds are chiefly derived from 11β -hydroxyandrostenedione, a substance of unknown physiological significance. This short summary of the precursors of the urinary neutral 17-OS conjugates indicates their complex and diverse origins.

A method was set up to measure the most important sulphate and glucuronide conjugates of these neutral 17-oxosteroids in urine. This method, adapted from the method of O'Kelly (1968), permits the measurement of individual 17-OS as the separated steroid glucuronide and sulphate fractions of 24-hour urine specimens.

B. MATERIALS AND METHODS FOR DETERMINATION OF URINARY
17-OXOSTEROID CONJUGATES.

Reagents.

Anhydrous sodium sulphate, ammonium sulphate, potassium dihydrogen orthophosphate and sodium chloride were all BDH analar grade reagents.

The alumina, Woelm neutral activity grade 1, was obtained from Camlab, Cambridge.

The gas chromatographic column packings (3% QF-I by weight on 100/120 mesh Gas-Chrom Q and the 3% NGS by weight on 100/120 mesh Gas-Chrom S), the trimethylchlorosilane and the dimethylchlorosilane were obtained from Applied Science Laboratories, P.O. Box 440, State College, Pennsylvania, U.S.A.

Radioactive compounds.

The 7α - ^3H -pregnenolone (specific activity 5 Ci/mmol), the 7α - ^3H -pregnenolone sulphate (specific activity 16 Ci/mmol), the 7α - ^3H -dehydroepiandrosterone (specific activity > 10 Ci/mmol) and the 7α - ^3H dehydroepiandrosterone sulphate (specific activity 1 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks.

Solvents.

Ethanol and methanol (Burroughs Analar grade) were used without further purification.

Acetone, ether and toluene (BDH analar grade) were used without further purification.

Ethyl acetate and benzene were obtained from Reeve Angel Scientific Ltd. These solvents come in 500 ml packs and are specifically purified for chromatography; the g.l.c. purity being better than 99.7%. These solvents were used without further purification.

Hexane (BDH analar grade) was washed three times with 100 ml of conc. H_2SO_4 per litre for one hour on a mechanical shaker, followed by two washes with 100 ml of 2 N NaOH per litre, and finally with water until neutral. It was then dried with calcium chloride and distilled through a fractionating column.

Diethylamine (BDH analar grade) was stored over KOH pellets.

Choice of internal standard.

The choice of an internal standard is subject to the following considerations:

- a) It must have a detector response which is linearly related to the amounts present.
- b) The ratio of its amount to the steroids being measured must remain constant throughout the analytical procedure.
- c) It must be separable by gas-liquid chromatography from the other steroids to be investigated.
- d) The position at which it emerges on the gas chromatographic trace must be free from other contaminating peaks.

- e) It must not occur in urine.
- f) It must be as similar as possible in chemical structure to the compounds under investigation.
- g) It must be eluted from the alumina column in the same fraction as the other steroids under investigation.

Pregnenolone appeared to fulfil these requirements. It is not present in urine in detectable quantities; it is eluted in the same fraction as the 17-oxosteroids from the alumina columns; the ratios of peak heights obtained by g.l.c. for the control solutions and for the processed solutions were constant (Table 4a,b); it is easily separable from the 17-OS by gas liquid chromatography on a variety of columns and is free of extraneous contamination. Its structure is very similar to DHA, which is the most labile of the 17-oxosteroids. Under the conditions employed, no significant degree of breakdown has been observed for any of the steroids investigated.

Preparation of β -glucuronidase from limpet visceral sacs.

Limpets were collected, the visceral sacs were removed and homogenized in water. Four volumes of ice-cold acetone were added with continuous stirring. The mixture was allowed to stand for thirty minutes and was then filtered through Whatman No. 1 paper in a Buchner funnel. This material was resuspended in the same volume of ice-cold acetone, allowed to stand for a further 30 minutes and again filtered. It

was then washed with acetone on the filter paper until the filtrate was colourless. The resulting acetone powder was air dried overnight and then placed in a desiccator to remove the last traces of acetone.

The β -glucuronidase content of the powder was assayed by the method of Fishman (1963), using phenolphthalein glucuronide as a substrate. The glucuronidase activity was expressed as the number of μ g of phenolphthalein liberated by 1 g of powder under the conditions of the assay. The powder prepared in this manner was found to contain approximately $\frac{1}{2}$ -million units of β -glucuronidase activity per gram.

Collection and Storage of Urine.

Specimens for gas-liquid chromatography and chemical analysis were collected and stored in the cold room. To minimize any alterations which have been found to occur with repetitive thawing and freezing, the urines were stored in 200 ml aliquots. Specimens for 17-oxosteroid analysis at normal urine pH undergo little or no change after one month in the cold. Some hydrolysis of steroids has been reported to occur during extended storage in the deep freeze, which is intensified by thawing and refreezing (Patti & Stein, 1964).

Solvolysis of 17-oxosteroid sulphates.

Steroid sulphates were hydrolysed by the method of Burstein & Lieberman (1958). Pregnenolone sulphate (40 μ g) was added as an internal standard to 25 ml of a complete

24-hour urine. Na Cl was added to this to give a 20% (w/v) solution and the pH was adjusted to 1 by the addition of 4N.H₂SO₄. The solution was then extracted twice with an equal volume of ethyl acetate and the combined extracts incubated, for approximately 20 hours at 37°C. The extract was then washed two or three times with 0.1 volume N.NaOH until no further pigments were removed, and finally three times with 0.1 volume distilled water. The washed extract was dried by passing through anhydrous sodium sulphate on a filter and evaporated to dryness under a stream of air.

Hydrolysis of 17-steroid glucuronides.

Steroid glucuronides were hydrolysed by the following method.

A suitable steroid glucuronide for use as an internal standard is not readily available. Accordingly, 50 µg pregnenolone was added to 25 ml of urine from a complete 24-hour specimen. In order to avoid inhibitors of β-glucuronidase which are present in the urine, the glucuronide conjugates were extracted by the method of Edwards, Kellie & Wade (1953). Ammonium sulphate was added to give a 50% (w/v) solution and the pH was adjusted to less than 5 by the addition of glacial acetic acid. The solution was then extracted three times with equal volumes of ether/ethanol, 3:1, and the combined extracts evaporated under reduced pressure. The residue was leached twice with 10 ml of ethanol to remove the internal standard

and the glucuronides, and then filtered through Whatman No. 1 paper. This is necessary, since ammonium sulphate inhibits β -glucuronidase. The combined ethanol extracts were evaporated under a stream of air. The residue was mixed with 5 ml of the following enzyme preparation: an acetone dried powder containing β -glucuronidase from the common limpet was suspended in 0.5M acetate buffer (pH 4.7), containing 0.05M sodium or potassium phosphate, to give 20,000 enzyme units/ml. The phosphate inhibits any steroid sulphatase present (Roy, 1956). The mixture was incubated at 37°C for 2 days. After hydrolysis, 20 ml of distilled water were added and the liberated steroids were extracted twice with an equal volume of ether/ethyl acetate (1:1). The combined extract was washed two or three times with 0.1 volume N.NaOH until no further pigments were removed and finally three times with distilled water to remove the alkali, dried by passage through anhydrous sodium sulphate on a filter and evaporated under a stream of dry air.

From this stage, the residues containing steroids from the sulphate and glucuronide fraction are treated in the same way.

Alumina column chromatography.

Columns (10 x 5 mm) containing 1 g alumina weakened by addition of 4% water were prepared in dry benzene. They were then tested with a mixture of 50 μ g of each of the following steroids in 3 ml of dry benzene: androsterone (A),

11-oxo-A, 11 β -OH-A, aetiocholanolone (E), 11-oxo-E, 11 β -OH-E, dehydroepiandrosterone, epiandrosterone and pregnenolone (the last mentioned is the g.l.c. internal standard). The test mixture was transferred to the column and washed in a further 3 ml dry benzene. The column was washed with 7 ml benzene and eluted with 25 ml 1.4% ethanol in benzene. The last solvent should remove all 17-oxosteroids from the column. This was checked by comparison on the g.l.c. after TMSE formation, with a similar mixture subjected to etherification but without putting through the alumina column. When the correct volume of eluting solvent was determined for the batch of alumina in use, the urine extract was transferred to the alumina column with 2 x 3 ml benzene, and the column washed and eluted as already described. The 1.4% ethanol in benzene elutes both 11-oxy- and 11-deoxy-17-OS (see Heyns & de Moor, 1965).

Preparation of trimethylsilyl ethers (TMSE).

The trimethylsilyl ethers were prepared by the method of Thomas & Walton (1968). The steroid residue in a 3 ml conical tube was further dried in vacuum for several hours. Traces of water prevent quantitative preparation of TMSE (Lau, 1966). Five ml hexane containing 0.7 ml trimethylchlorosilane were added to 5 ml hexane containing 0.4 ml diethylamine, shaken vigorously and centrifuged. About 0.4 ml supernatant solution was added to the dry steroid residue, the tube was stoppered and left overnight. The reaction mixture was then evaporated with the tube in a

vacuum desiccator. Trimethylsilyl ethers of the steroids in the residue were dissolved in hexane to give a concentration of pregnenolone internal standard of about 0.5 $\mu\text{g}/\mu\text{l}$. The insoluble ammonium chloride was centrifuged down, since traces interfere with subsequent gas chromatography. Along with the unknown urine samples, residues containing equal amounts of 17-O5 and pregnenolone, were put through the preparation procedure, to check TMSE formation and correct peak height ratios.

Gas-liquid chromatography.

The instrument used in this present work was a Pye Series 106, Model 6, Automatic Analytical Gas Chromatograph equipped with an automatic solids injection system, injection port heater and a flame ionisation detector. Glass columns, spiral-shaped, 2.23 metres long and with an internal diameter of 2 mm, were specially made by Aimer Products Ltd., 56-58 Rochester Place, London, N.W.1. A Leeds and Northrup Speedomax W Recorder was employed with a chart speed of 10 in/h. Purified nitrogen was used as the carrier gas, with an inlet pressure of approximately 40 psi, giving a flow rate of 45 ml/min. The hydrogen and air flow rates were 50 ml/min and 500 ml/min respectively. All flow-rates were measured at the detector outlet by means of a soap-bubble flow-meter.

Packing of columns.

Before packing the columns with the precoated support,

both the glass columns and the glass wool to be used were treated overnight with 5% (w/v) dimethyldichlorosilane in toluene; then washed with methanol and thoroughly dried. This process removes any 'active sites' on the glass wool or the internal wall of the glass column which might absorb the polar steroid molecules causing loss of sample or tailing of the chromatographic peak.

The glass column was then packed as follows: one end of the column is plugged with silanized glass wool and attached to a relatively high capacity vacuum pump. The packing can then be carefully drawn into place through a suction procedure. During the packing process, the column should be tapped firmly with a rubber-covered rod to ensure dense and even packing. It is also possible to use a mechanical vibrator, but excessive vibration should be avoided, since the packing may be partially shattered. Before use, a conditioning period is necessary for all newly packed columns. To this end both types of columns (NGS and QFI) were heated to 250⁰C in a stream of nitrogen for 24 hours. This pretreatment of the column ensured the removal of volatile products which may contaminate the detector and prevent a stable recorder baseline.

Introduction of samples into the gas chromatograph.

Solutions of the TMSE derivatives of the steroids were prepared in hexane, resulting generally in a concentration of 0.1 - 1.0 µg of steroid per µl of hexane. Depending on the

concentration, volumes varying between 1 - 20 μ l of the hexane solution were injected by means of a syringe into the glass sample holders (maximum volume 40 μ l), which were heated by the magazine heater for approximately 20 minutes, depending on the volume injected. When all the solvent had evaporated, the magazine containing the sample holders was transferred into the glass casing on top of the column. A signal from the auto-solids injector controller actuated the mechanism which pushed a sample holder into the heated zone at the column inlet. The sample was quickly evaporated and flushed on to the column in a minimum of carrier gas and the used holder was ejected into the glass receiver. The injection sequence could be repeated at preset time intervals varying between ten and ninety minutes, for up to 36 sample holders.

Cleansing of the glass sample holders.

The sample holders were washed several times with the purified hexane and then heated to about 300°C in an oven. After cooling, they were immersed overnight in a 5% solution (w/v) of dimethyldichlorosilane in toluene. They were then rinsed twice with toluene, twice with methanol and dried in a moderately warm oven.

CALCULATION OF RESULTS.

The peak heights of all the steroids were measured in mm. This was found to be simpler, quicker and just as accurate as

measuring peak areas. Standard solutions containing equal amounts of all the 17-oxosteroids to be measured, including the internal standard, were chromatographed at the beginning, the middle and the end of every batch of urinary analyses to monitor retention times and column performance. Their peak heights were also measured. The following formula was used to calculate the number of mg of each steroid in the 24-hour urine specimen.

$$\begin{array}{l} \text{mg of steroid in} \\ \text{24-hour urine sample} \end{array} = \frac{a}{b} \times \frac{d}{c} \times \frac{e}{g} \times \frac{f}{1,000}$$

Where:

- a = peak height of 17-OS in the analytical specimen;
- b = peak height of pregnenolone in the analytical specimen;
- c = peak height of 17-OS in the standard;
- d = peak height of pregnenolone in the standard;
- e = number of µg of pregnenolone added initially to the urine sample;
- f = volume of 24-hour urine collection;
- g = volume of aliquot taken for analysis.

All the urinary values for the conjugated 17-OS are expressed in terms of the concentration of free steroid per 24-hour urine specimen, unless otherwise stated.

C. STATISTICAL PROCEDURE.

Student's 't' test.

This is a test of significance and is used to compare the means of two independent samples and, from the results, conclude if the samples are from the same or different populations. For example, comparison of the steroid values obtained for normal subjects and for porphyric patients gives the 't' value:

$$'t' = \frac{(\bar{x} - \bar{y})}{S\sqrt{\left(\frac{1}{n} + 1\right)}}$$

where:

\bar{x} is the mean of the normal subjects;

\bar{y} is the mean of the porphyric patients;

S^2 is the estimate of the variance obtained

from normal subjects, where $S^2 = \frac{1}{N_x - 1} \sum_{i=1}^{N_x} (x_i - \bar{x})^2$

and N_x is the number of normal subjects. Having calculated 't' and the number of degrees of freedom (d.f.) by the formula

$$d.f. = \frac{n_x + n_y}{2} - 1$$

where:

n_x is the number of samples in the first group; and

n_y is the number of samples in the second group;

the statistical significance of 'p' value is obtained

from the 't-distribution' tables. The conditions

which must be satisfied to make the Student's 't' test a powerful one and, in fact, before any confidence can be placed in any probability statement obtained by the use of the 't' test, are at least the following:

- 1) The values must be independent.
- 2) The values must be drawn from normally distributed populations.
- 3) These populations must have the same variance or, in special circumstances, they must have a known ratio of variance.

Correlation coefficient.

In measuring the association between two normal variables, it is convenient first to generalize the variance estimate used for measuring the variability of a single variable. Suppose that we have observed the variables x and y on n individuals, obtaining values $x_1, y_1; x_2, y_2; \dots; x_n, y_n$.

The variance of x and y are estimated by

$$s_x^2 = \frac{\sum (x - \bar{x})^2}{n - 1}$$

$$s_y^2 = \frac{\sum (y - \bar{y})^2}{n - 1}$$

A simple first measure of the association between x and y is the covariance which is estimated by

$$c_{xy} = \frac{\sum (x - \bar{x})(y - \bar{y})}{n - 1}$$

The covariance estimate is a simple measure of association but it is not easy to interpret because it depends on the scale on which the variables are measured. A more useful quantity is the correlation coefficient estimated by

$$r = \frac{\text{Covariance}}{(\text{S.D. of } x)(\text{S.D. of } y)} = \frac{C_{xy}}{S_x S_y}$$

It may be shown that the correlation coefficient never exceeds 1 in numerical value, i.e. $-1 \leq r \leq 1$.

When $r = 1$, the sample relationship is a straight line with x and y increasing together and, when $r = -1$, the relationship is again a straight line with y now decreasing when x increases. Intermediate values of ' r ' are less easy to interpret, but it can be accepted that the values of ' r ' near to $+1$ or -1 indicate a close relationship with little departure from linearity.

D. EVALUATION OF THE RELIABILITY OF THE METHOD FOR
DETERMINATION OF THE URINARY 17-OXOSTEROIDS.

The 17-OS were separated and quantitated as their trimethylsilyl ether derivatives on the selective phases NGS and QF-1. Epiandrosterone TMSE was not separated from DHA TMSE on the NGS column. This metabolite (Epi-TMSE), especially the glucuronide conjugate is usually a minor constituent of the urinary 17-OS and, if its estimation is desired, the liquid phase QF-1 must be used (Fig. 21). Similarly, it was not possible to wholly separate 11-oxoandrosterone, 11-oxo Δ^5 -cholestanolone, 11-hydroxyandrosterone and 11-hydroxy Δ^5 -cholestanolone on the stationary phase QF-1, and it was necessary to use NGS (Fig. 22). By far the larger fraction of these fore-mentioned $C_{19}O_3$ steroids are excreted as glucuronide conjugates, thus the NGS phase was used to separate and quantitate the glucuronide fraction of the urinary 17-OS. Similarly, since the sulphates of DHA and epiandrosterone are quantitatively more important than their glucuronide conjugates, the QF-1 liquid phase was used for separation and quantitation of the sulphate fraction.

Complete TMSE formation of the 17-OS was demonstrated on both QF-1 and NGS stationary phases. On both of these selective phases the TMSE had shorter retention times than their parent alcohols. Using the pure 17-OS mixture, no

GLC separation of a standard mixture
of eight 17-oxosteroids and the internal
standard

The peaks are identified as follows ;

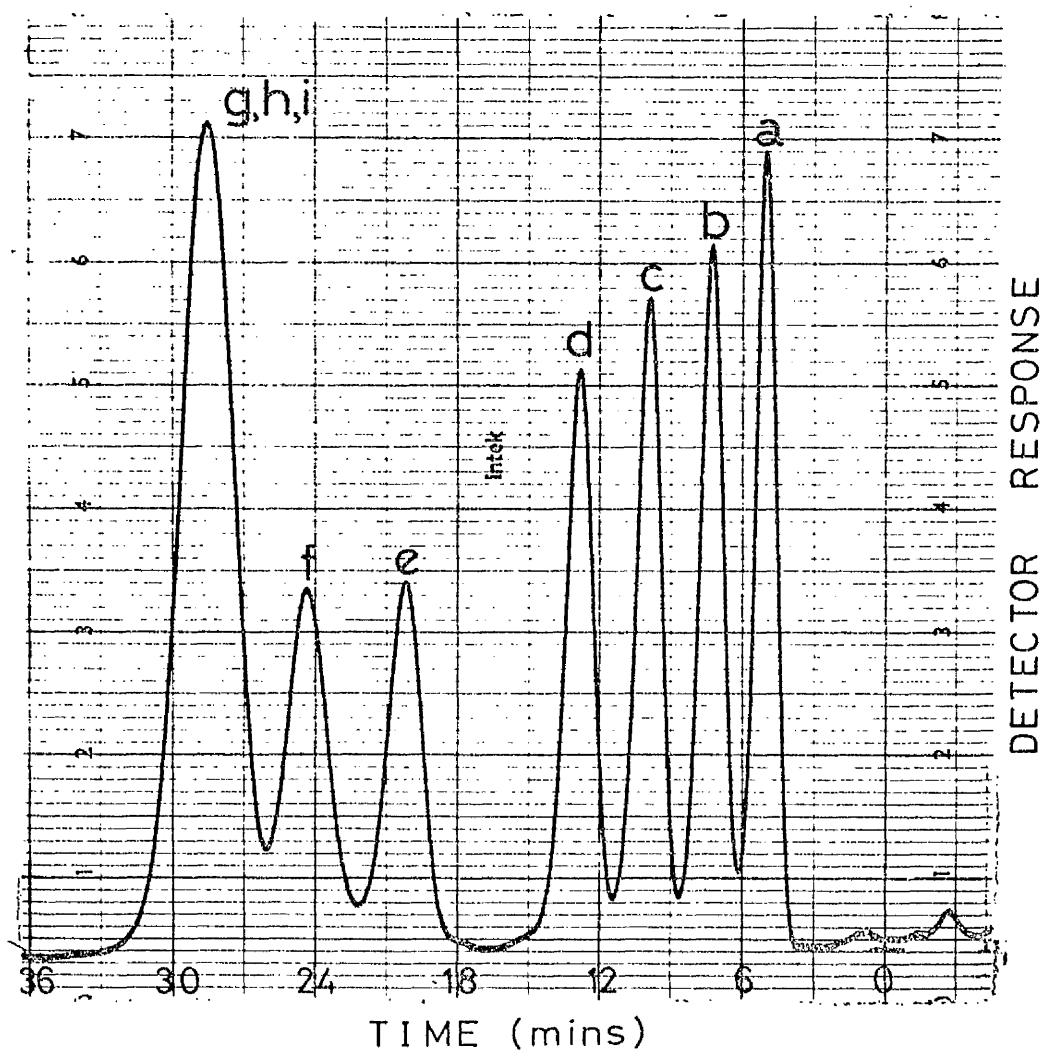
a	=	Androsterone
b	=	Aetiocholanolone
c	=	Dehydroepiandrosterone
d	=	Epiandrosterone
e	=	Pregnenolone (internal standard)
f	=	11-oxoandrosterone
g	=	11-oxoaetiocholanolone
h	=	11 β -hydroxyandrosterone
i	=	11 β -hydroxyaetiocholanolone

These letters are used to identify the respective steroid peaks in all the g.l.c. tracings shown in this thesis.

Figure 21.

GLC separation of a standard mixture
of 17-oxosteroids

Column 3%QF1 ; temp 169°C ; N₂ flow rate 50ml/min

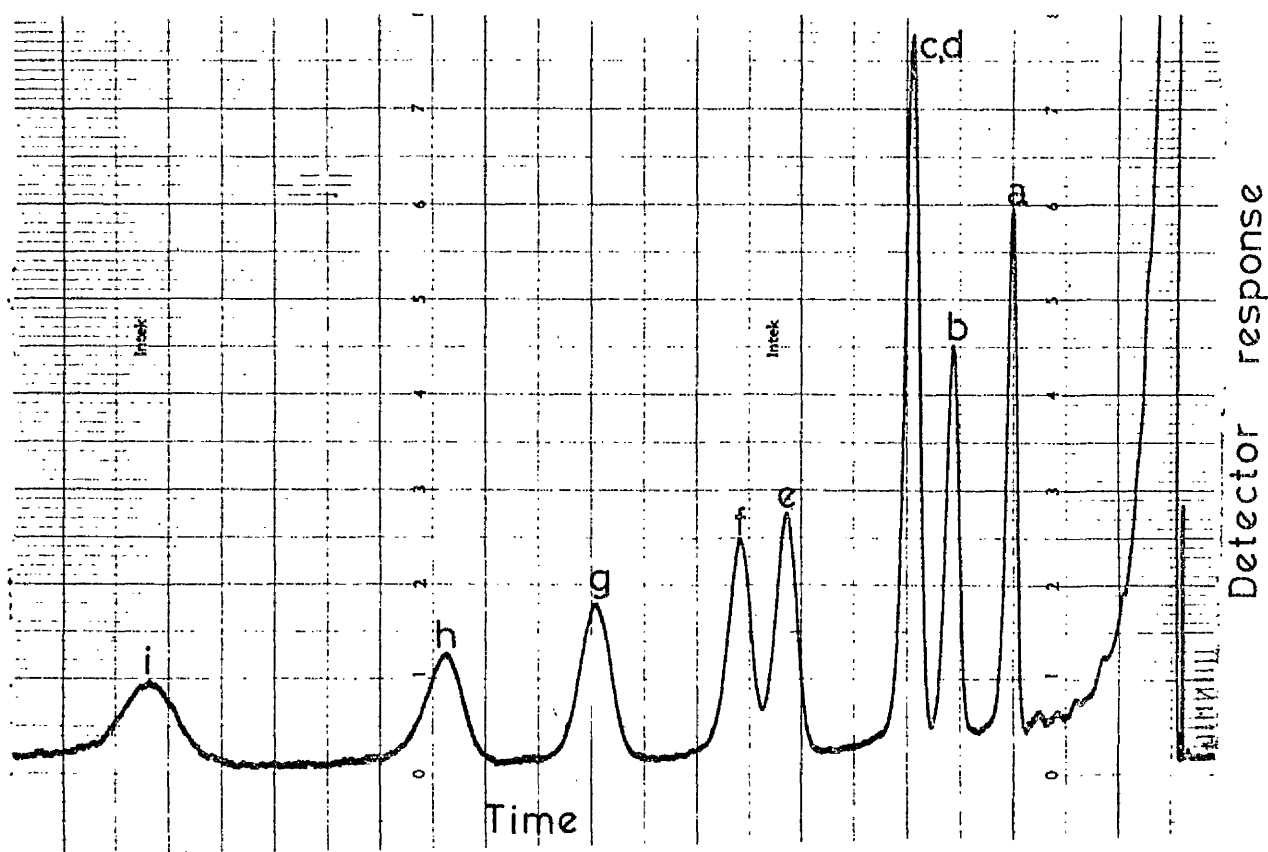


11-Oxo-aetiocholanolone (g), 11-hydroxy-aetiocholanolone (i) and 11-hydroxyandrosterone (h) are not separated on the QF1 column.

Figure 22

GLC separation of a standard mixture of 17-oxosteroids

Column 3% NGS ; temp 200°C ; N₂ flow rate 45ml/min



Epiandrosterone (d) and dehydroepiandrosterone (c) are not separated on the NGS column.

free steroids could be detected after derivative formation.

The response of the detector to the TMSE derivatives of the individual 17-OS to the response of the pregnenolone TMSE derivative was satisfactory. A straight line was obtained when the ratios of the masses of individual 17-OS to pregnenolone (0.1 to 10) were plotted against the ratios of the corresponding TMSE derivative peak heights (Fig. 23).

The retention time of individual TMSE derivatives relative to the retention time of the internal standard pregnenolone TMSE, was determined throughout the study. Although the g.l.c. conditions did not always remain constant from one series of analyses to another, the relative retention times of the TMSE derivatives were found to be constant for both the authentic and the urinary steroids (Table 2).

It was important to determine the value of each individual steroid derivative and its constancy over a period of time. Reference standard mixtures containing equal amounts of the eight 17-OS and the internal standard were chromatographed numerous times over the period of the study. The response to each steroid, measured as the ratio of its peak height to the peak height of an identical amount of pregnenolone, is shown in Table 3.

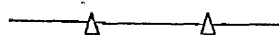
When an internal standard is used, it is necessary that the ratio of its amount to the steroids being measured remains constant throughout the analytical procedure. To establish whether this was so, aliquots were taken from a solution containing the sulphate esters of androsterone,

Calibration of the detector response for 17-oxosteroids

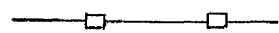
The ratios of the TMSE 17-OS derivative peak heights to the peak height of pregnenolone TMSE derivative were plotted against the ratios of the masses of the corresponding individual free 17-OS to the mass of free pregnenolone. Straight lines were obtained for all the plots and thus the mass of the free steroid is directly proportional to the peak height of its TMSE derivative. The plots of the ratios of the individual 17-OS to pregnenolone are represented by the following symbols:-



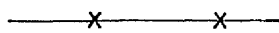
= Androsterone



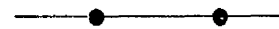
= Aetiocholanolone



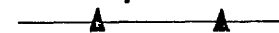
= Dehydroepiandrosterone + epiandrosterone



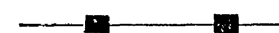
= 11-oxoandrosterone



= 11-oxoaetiocholanolone



= 11β-hydroxyandrosterone



= 11β-hydroxyaetiocholanolone

Calibration curves for 17-oxosteroids

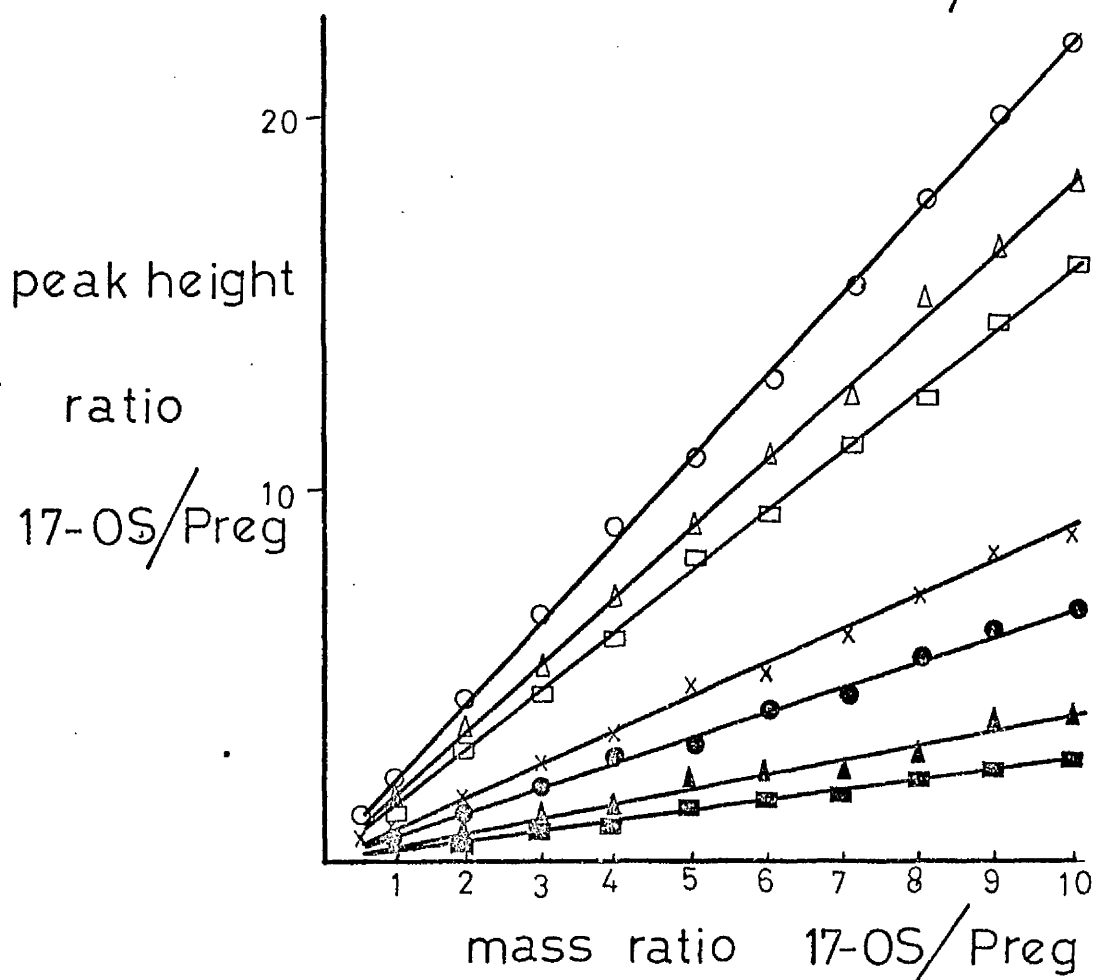
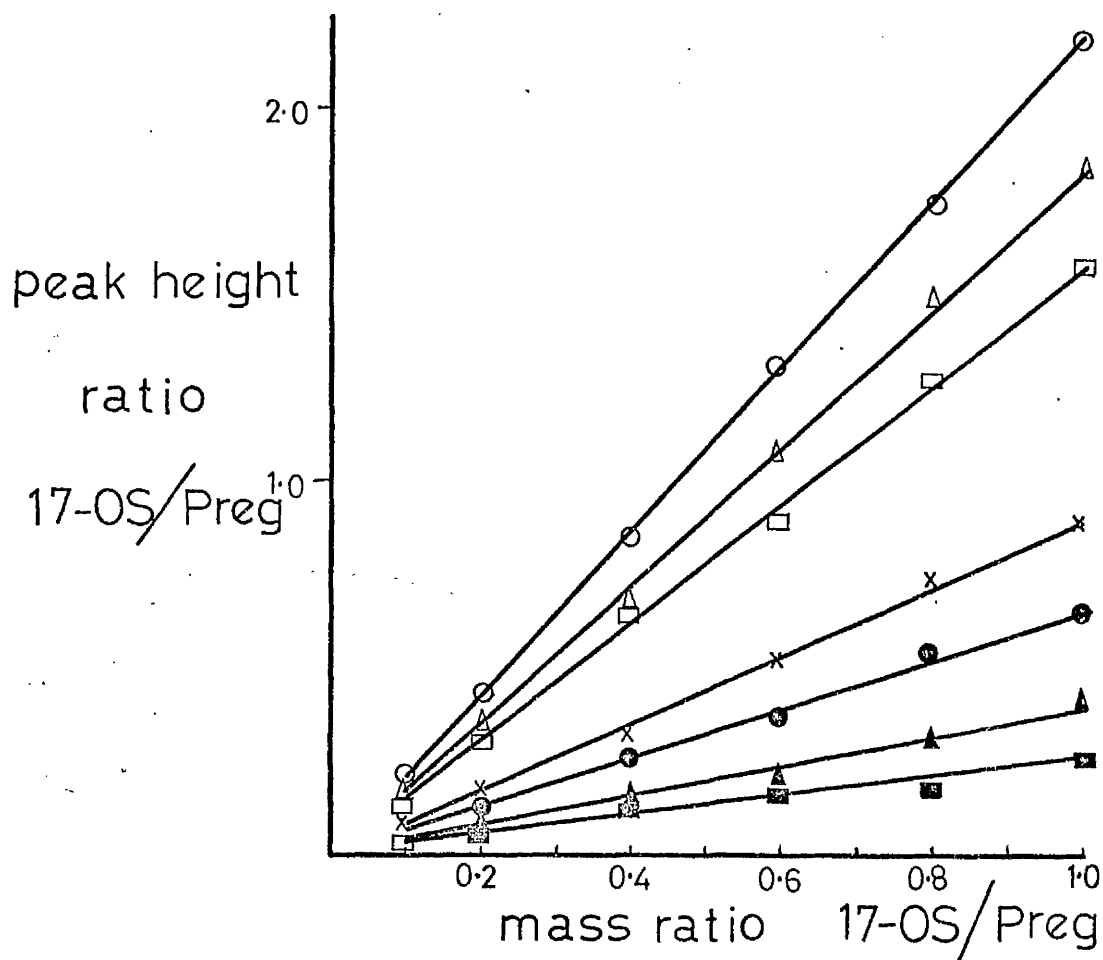


Table 2.

RETENTION TIMES OF THE 17-OS DERIVATIVES RELATIVE
TO PREGNENOLONE TMSE

STERIODS	AUTHENTIC STEROIDS			URINARY STEROIDS			COLUMN
	n	x	S.D.	n	x	S.D.	
A	30	0.499	0.004	30	0.499	0.004	
E	30	0.573	0.004	30	0.574	0.004	
DHA	30	0.659	0.005	30	0.659	0.006	3%
EPI	30	0.756	0.007	30	0.756	0.007	QF-1
A	30	0.434	0.003	25	0.435	0.003	
E	30	0.578	0.003	25	0.579	0.004	
DHA + EPI	30	0.686	0.005	25	0.686	0.005	
11-OA	30	1.116	0.006	25	1.116	0.006	3%
11-OE	30	1.453	0.014	25	1.456	0.015	NGS
11-OHA	30	1.817	0.018	25	1.815	0.017	
11-OHE	30	2.464	0.034	25	2.462	0.034	

n ~ number of determinations

x ~ mean relative retention time

S.D. ~ standard deviation

Table 3.

GAS CHROMATOGRAPHIC RESPONSE TO 17-OS MEASURED AS
PEAK HEIGHTS AND REFERRED TO PREGNENOLONE AS STANDARD

STEROIDS	n	MASS RATIO	PEAK HEIGHT RATIO x	S.D.
A	25	1	2.201	0.032
E	25	1	1.830	0.028
DHA + EPI	25	2	2.860	0.047
11-DA	25	1	0.900	0.019
11-OE	25	1	0.666	0.023
11-OHA	25	1	0.412	0.028
11-OHE	25	1	0.289	0.021

n - number of determinations

x - mean

S.D. - standard deviation

aetiocholanolone, DHA, epiandrosterone and pregnenolone. Some of these were solvolyzed and used as controls, and the remainder were put through the steps of the method. The ratios of peak heights obtained by g.l.c. on the QF-1 column for the control solutions and for the processed solutions were compared and the results are shown in Table 4a. These data show that there is little change in the peak height ratios throughout the procedure for quantitation of the sulphates. The suitability of pregnenolone as an internal standard for the glucuronide fraction was demonstrated by similar means (Table 4b). In this case, the reference mixture was used as the control, as the 17-OS glucuronides were unobtainable. The TMSE derivatives were chromatographed on the NGS column.

Specificity.

The specificity of the method depends upon the elimination of non-steroidal impurities and the adequate separation of steroids with similar chromatographic properties which may be present in the urine. The use of the alumina column ensures preferential isolation of the 17-OS. Evidence for the lack of interference from solvents and reagents was obtained by the analysis of distilled water and added steroids (Table 5a,b). The urinary steroid TMSE were identified by their retention times relative to pregnenolone TMSE, which were identical with authentic standard TMSE derivatives (Table 2). When

Table 4a.

SUITABILITY OF PREGNENOLONE SULPHATE AS AN INTERNAL STANDARD

		RATIO A Preg.	RATIO E Preg.	RATIO DHA Preg.	RATIO EPI Preg.
	n				
CONTROL	10	2.221	2.036	1.840	1.623
	S.D.	0.060	0.060	0.050	0.050
TEST	10	2.200	2.015	1.763	1.599
	S.D.	0.060	0.070	0.060	0.050

n - number of determinations

S.D. - standard deviation

Table 4b.

SUITABILITY OF PREGNENOLONE AS AN INTERNAL STANDARD

		<u>A</u>	<u>E</u>	<u>DHA + EPI</u>	<u>11-OA</u>	<u>11-OE</u>	<u>11-OHA</u>	<u>11-OHE</u>
	n	Preg.	Preg.	Preg.	Preg.	Preg.	Preg.	Preg.
CONTROL	8	2.191	1.839	2.852	0.909	0.670	0.408	0.295
	S.D.	0.070	0.070	0.100	0.030	0.030	0.020	0.009
TEST	8	2.103	1.796	2.883	0.919	0.594	0.387	0.291
	S.D.	0.072	0.071	0.121	0.053	0.036	0.024	0.011

n = number of determinations

S.D. = standard deviation

Table 5.

ANALYSIS OF DISTILLED WATER AND ADDED STEROIDS

- a) Recovery of A, E, DHA, EPI added to 25 ml Water by Sulphate Procedure.

Steroid Added	μg	n	μg Measured Mean	% Recovery	S.D.
A	50	8	49.4	98.8	2.9
E	50	8	51.1	102.2	2.5
DHA	50	8	51.6	103.2	1.9
EPI	50	8	50.3	100.6	2.0

- b) Recovery of A, E, DHA, EPI, 11-OA, 11-DE, 11-OHA, 11-OHE added to 25 ml Water by Glucuronide Procedure.

Steroid Added	μg Added	n	μg Measured Mean	% Recovery	S.D.
A	50	12	49.2	98.4	4.0
E	50	12	51.8	103.6	3.5
DHA + EPI	100	12	102.3	102.3	2.2
11-OA	50	12	50.2	100.4	3.8
11-DE	50	12	49.8	99.6	3.6
11-OHA	50	12	51.2	102.4	4.2
11-OHE	50	12	51.6	103.2	4.3

n - number of determinations

S.D. - standard deviation

authentic steroids were added to the urine for recovery studies, no new peaks were formed; only the increases of the respective peak areas were observed. Similarly, if no internal standard was added to the urine, no peaks were found on either gas chromatographic trace, which would have contributed to the peak height of pregnenolone TMSE.

The shapes of the peaks in both analytical and standard were identical, with no evidence of contamination from unknown peaks.

Finally, sulphate and glucuronide fractions were chromatographed on both QF-1 and NGS columns, and essentially identical results obtained (Table 6a,b).

Precision.

The precision of the method was determined by repeated estimations on three urine pools. In Tables 7 and 8, the results obtained in the analyses of these pools are given. From these values it can be seen that the precision of the methods is similar for all the steroids determined covering a wide concentration range.

Accuracy.

The accuracy of the method was assessed by the addition of known amounts of the free steroids or the steroid sulphates to pooled urine or distilled water, processing the aliquots by the methods described and calculating their recoveries. The results are presented in Tables 5a and b, 9, 10 and 11.

In all the experiments, the mean percentage recovery

Table 6.

REPLICATE ANALYSES OF A URINE POOL. THE FINAL EXTRACTS WERE CHROMATOGRAPHED
ON BOTH QF-1 AND NGS COLUMNS (mg FREE STEROID/LITRE OF URINE POOL)

a) Glucuronide Fraction from Urine Pool.

Column	n	A	E	DHA	EPI	11-OA	11-OE	11-OHA	11-OHE
3% QF-1	14	2.08	1.87	0.36	0.12				
S.D.		4.8%	5.3%	6.1%	6.3%				
3% NGS	14	2.09	1.77		0.46	0.23	1.27	0.59	0.62
S.D.		5.2%	5.5%		6.5%	8.7%	6.3%	6.5%	7.9%

b) Sulphate Fraction from Urine Pool.

Column	n	A	E	DHA	EPI
3% QF-1	14	1.05	0.36	2.72	0.57
S.D.		5.7%	8.3%	3.7%	5.3%
3% NGS	14	1.12	0.34		3.18
S.D.		6.9%	5.9%		3.8%

n - number of determinations.

S.D. - standard deviation %

Table 7.

Pool	n	A	E	DHA	EPI
X	8	1.06	0.34	2.65	0.61
	S.D.	4.7%	8.8%	3.0%	6.5%
X	14	1.05	0.36	2.72	0.57
	S.D.	5.7%	8.3%	3.0%	6.5%
X	6	1.08	0.32	2.58	0.59
	S.D.	7.4%	9.4%	3.9%	6.8%
X	6	1.16	0.32	2.69	0.54
	S.D.	4.3%	7.1%	4.2%	6.2%
Y	8	2.57	1.28	3.81	1.53
	S.D.	5.1%	4.8%	2.9%	4.2%
Z	8	3.61	2.86	4.01	2.98
	S.D.	4.2%	3.5%	2.3%	2.4%

Table 7. Replicate analyses of three pools. Pregnenolone sulphate was added as internal standard and the steroids were hydrolyzed and extracted as described for steroid sulphate estimation. The final extracts were chromatographed on a 3% QF-1 column. The results are expressed as mg of free steroid per litre of urine pool.

n -- number of determinations

S.D. -- standard deviation %

Table 8.

POOL	n	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE
X	14	2.09	1.77	0.46	0.23	1.27	0.59	0.62
	S.D.	6.2%	5.2%	6.5%	8.7%	6.3%	6.8%	8.0%
Y	8	4.04	3.79	1.01	0.18	0.78	0.99	0.63
	S.D.	4.7%	4.5%	5.9%	5.6%	6.8%	5.0%	6.4%
Z	8	4.65	4.02	1.89	0.29	1.18	1.19	0.79
	S.D.	4.1%	5.6%	5.3%	6.9%	9.3%	7.6%	6.3%

Table 8. Replicate analyses of three urine pools. Pregnenolone was added as internal standard and the steroids were hydrolyzed and extracted as described for steroid glucuronide estimation. The final extracts were chromatographed on a 3% NCS column. The results are expressed as mg of free steroid per litre of urine pool.

n - number of determinations

S.D. - standard deviation %

Table 9.

RECOVERY OF 17-OS SULPHATES ADDED TO 25 ml ALIQUOTS
OF URINE POOL

	A	E	DHA	EPI
No. of Analyses	8	8	8	8
µg of steroid present	27.0	8.0	67.0	13.5
µg added*	33.9*	33.9*	33.7*	33.9*
Mean µg measured	60.1	42.3	100.0	48.2
% Recovery	97.6%	101.2%	97.9%	102.4%
S.D.	4.8	5.7	3.9	6.1

Recovery of AS, ES, DHAS and EPIS added to urine. Pregnenolone sulphate was added as an internal standard. The final extracts were chromatographed on a 3% QF-1 column.

*Calculated amount of free steroid added

Table 10.

RECOVERY OF INCREASING AMOUNTS OF DHAS ADDED TO 25 ml
 ALIQUOTS OF THE URINE POOL

SAMPLE	n	μ g DHA Present	μ g DHA Added *	Mean μ g DHA Measured	Mean % Recovery	S.D.
1	2	67	0	67	-	-
2	2	67	0.7	67.4	57.1%	7.9
3	2	67	3.4	70.1	91.2%	6.1
4	2	67	6.8	73.5	95.6%	4.2
5	2	67	16.9	83.7	98.8%	3.8
6	2	67	33.7	101.1	101.2%	2.6
7	2	67	67.5	134.4	99.8%	3.1

Pregnenolone sulphate was added as internal standard. The final extracts were chromatographed on a 3% QF-1 column.

*Calculated amount of free steroid added

Table 11.

RECOVERIES OF 17-OS ADDED TO 25 ml ALIQUOTS OF URINE POOL

	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE
No. of Analyses	8	8	8	8	8	8	8
µg present	52.2	44.2	11.5	5.8	31.8	14.8	15.5
µg added	50	50	50	50	50	50	50
mean µg measured	101.5	92.5	57.1	50.9	85.3	59.8	59.4
mean recovery	98.6%	95.6%	91.2%	50.2%	107.0%	90.0%	87.8%
S.D.	2.6	3.2	5.3	6.7	7.2	6.9	6.6

Recovery of free 17-OS added to urine pool. Pregnenolone was added as internal standard and the steroids were hydrolyzed and extracted by the method described for the steroid glucuronide estimation. The final extracts were chromatographed on 3% NGS.

relative to the internal standard was within the range 87.8 - 107.1 with a percentage standard deviation within the range 2.6 - 7.9, which is quite satisfactory.

The absolute recoveries of the internal standard were achieved by adding tritium labelled pregnenolone sulphate or tritium labelled pregnenolone to urine samples and estimating the recovery of radioactivity at the end of the method. The results are shown in Tables 12a and b. Similar absolute recovery experiments were repeated using 7α - ^3H -DHA and 7α - ^3H -DHAS. These results are also shown in Tables 12a and b.

The mean overall recovery for the internal standard, Preg. SO_4 , through the procedure described for determination of steroid sulphates, was 86.3%, with a standard deviation of 2.3. This was very satisfactory and agreed with the total mean percentage of radioactivity recovered when the experiment was repeated using ^3H -DHAS. This gave a total mean recovery of 91.3% and, once again, the precision was very good with a standard deviation of 3.0. Overall percentage recoveries for the procedure required for estimation of steroid glucuronides were not as high as those for the sulphate procedure. The mean recovery of added ^3H -pregnenolone was 70.6%, and for DHA 68.6%. This fall is to be expected when the additional steps involved in the glucuronide procedure are taken into consideration. Once again, the precision was very good with a standard deviation of 3.8 for the mean recovery of the pregnenolone.

Table 12a.

RECOVERY OF 7α - ^3H -PREGNENOLONE SULPHATE (122, 621 d.p.m.)
 AND 7α - ^3H -DHA SULPHATE (144, 420 d.p.m.) ADDED TO URINE

Steroid	n	Mean d.p.m.	% Recovery	S.D.
^3H -Preg. SO_4	10	105822	86.3%	2.3
^3H -DHA SO_4	20	104465	91.3%	3.0

The above table shows the total recovery of tritiated Preg. SO_4 and tritiated DHA SO_4 added to separate 25 ml aliquots of the urine pool. Pregnenolone SO_4 was added as internal standard and the steroids were hydrolysed and extracted by the method described for the steroid sulphate estimation.

n - number of determinations

S.D. - standard deviation

Table 12b.

RECOVERY OF $7\alpha\text{-}^3\text{H}$ -PREGNENOLONE (151, 620 d.p.m.) AND
 $7\alpha\text{-}^3\text{H}$ -DHA (216, 321 d.p.m.) ADDED TO URINE

Steroid	n	Mean d.p.m.	% Recovery	S.D.
^3H -Preg.	10	107044	70.6%	3.8
^3H -DHA	10	148396	68.6%	4.3

The above table shows the total recovery of tritiated pregnenolone and tritiated DHA added to separate 25 ml aliquots of the urine pool. Pregnenolone was added as internal standard and the steroids were hydrolyzed and extracted by the method described for the steroid glucuronide estimation.

n - number of determinations

S.D. - standard deviation

Sensitivity.

In the present method, 25 ml of urine were used in each analysis. The usual amount injected into the gas chromatograph and the usual setting of the attenuator allowed the measurement by planimetry of epiandrosterone TMS ether peak on the QF-1 column, when the concentration of this steroid was greater than or equal to 1 $\mu\text{g}/25$ ml urine. This peak was the last one of the quantitated steroids to emerge from the column, thus having a least favourable form for measurement of peak height. Similarly with the procedure for estimation of the glucuronide fraction, the lowest value which could be distinguished from zero for 11 β -hydroxy-aetiocholanolone, whose TMSE derivative emerged last from the column, was 1 $\mu\text{g}/25$ ml urine. The sensitivity of both procedures may be increased by using a larger fraction of the 24-hour urine specimen and less internal standard, or by increasing the volume of hexane solution of the final extract used for g.l.c. The gas-liquid chromatograph has the potential for a much greater sensitivity than that usually used, but the use of very high sensitivities puts great demands on the purity of the solvents. Thus, attempts were not made to further scale up the sensitivity of the method.

It can be concluded from all the forementioned investigations that these two procedures for the estimation of urinary 17-oxosteroid glucuronides and sulphates are very satisfactory. In order to check the continuing accuracy and precision of the two procedures throughout the study, a pooled urine sample of known 17-oxosteroid concentration was repeated in duplicate with each batch of analyses.

SECTION V

THE URINARY EXCRETION OF 17-OXOSTEROID

GLUCURONIDES AND SULPHATES BY NORMAL

MALES AND FEMALES

THE URINARY EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND
SULPHATES BY NORMAL MALES AND FEMALES.

A total number of seventy-five normal subjects were studied, consisting of thirty-eight males and thirty-seven females. They were mainly members of staff, friends and ambulant patients who were free from any disorders which might influence the levels of the 17-OS. Some of these disorders which have been reported to have an effect on the levels of 17-OS in the urine are, thyroid disease (Hellman et al., 1959), gout (Sonka et al., 1965), fever (Kappas and Palmer, 1965), obesity (Hendrikx et al., 1965; Lopez and Krehl, 1967), diabetes mellitus (Sonka and Gregorova, 1964; Charro-Salgado et al., 1968), breast cancer (Bulbrook, 1972), lung cancer (Rao, 1972) and other endocrine abnormalities (Lorraine and Bell, 1971). In the normal male group, the ages range from seventeen years through to sixty-five years. For comparative studies, this group of normal males was divided into smaller groups according to age, and the values for the individual males in their age groups along with the means and standard deviations for each group are shown in Tables 13(a - e). In the normal females studied, the ages ranged from eighteen years to forty-nine years. It has been reported by Bulbrook and Hayward (1969) that women using oral contraceptives excrete less urinary 11-deoxy-17-oxosteroids than women not using this form of contraceptive.

Table 13a

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL MALES (mg FREE STEROID/24 HOUR VOLUME)

GLUCURONIDES									SULPHATES				TOTAL
No.	Age	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI	
1	17	3.62	2.88	0.77	0.48	0.96	1.21	0.63	1.11	0.38	3.66	0.43	16.13
2	18	4.23	3.25	0.03	0.28	0.47	1.35	0.41	1.21	0.16	0.36	0.29	12.04
3	19	3.27	3.01	0.41	0.18	0.68	0.99	0.09	2.01	0.42	2.09	0.32	13.47
Range	17-19	3.27-4.23	2.88-3.25	0.03-0.77	0.18-0.48	0.47-0.96	0.99-1.35	0.09-0.63	1.11-2.01	0.16-0.42	0.36-3.66	0.29-0.43	12.04-16.13
Mean	18.0	3.71	3.05	0.40	0.31	0.70	1.18	0.38	1.44	0.32	2.04	0.35	13.88
S.D.	1.0	0.49	0.19	0.37	0.15	0.25	0.18	0.27	0.48	0.14	1.65	0.07	2.08

Table 13b

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL MALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	GLUCURONIDES							SULPHATES				TOTAL
		A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI	
4	20	5.61	3.73	0.34	0.10	1.55	1.77	1.26	1.32	0.42	1.23	0.29	17.62
5	21	3.24	2.77	0.02	0.24	0.26	0.71	0.26	0.70	0.27	0.29	0.32	9.06
6	21	5.04	4.65	1.09	0.46	1.11	1.21	0.69	2.86	0.80	4.89	0.66	23.45
7	22	3.94	6.04	0.08	0.25	1.41	0.71	0.76	0.43	0.19	0.20	0.16	14.17
8	23	6.89	5.77	0.98	0.51	1.09	2.02	0.51	3.16	0.51	3.61	0.72	25.77
9	24	10.80	7.17	0.36	0.53	0.88	1.39	0.00	0.87	0.17	1.10	0.22	23.49
10	24	10.85	7.19	1.00	0.26	1.06	1.32	0.46	2.88	0.29	3.02	0.41	27.74
11	25	2.04	2.28	0.34	0.48	0.65	0.67	0.72	0.91	0.25	3.15	0.36	11.85
12	25	4.25	5.81	0.44	0.24	1.08	1.45	0.52	3.08	0.45	5.69	0.71	23.72
13	26	4.02	4.50	0.11	0.17	1.43	1.27	0.93	1.30	0.93	0.88	0.75	16.29
14	26	2.80	1.78	0.10	0.15	0.87	1.37	0.41	0.66	0.05	0.34	0.15	8.68
15	27	6.41	5.02	0.06	0.42	1.02	1.63	0.47	0.24	0.08	0.21	0.14	15.70
16	29	4.02	4.27	1.69	0.32	0.94	1.47	1.03	3.74	0.94	6.11	0.76	25.29
17	29	8.83	7.14	4.44	0.41	1.19	2.16	0.00	1.18	0.24	3.27	0.22	29.08
18	29	3.48	0.69	0.63	0.21	0.18	0.71	0.00	0.69	0.08	0.80	0.16	7.63
Range	20-29	2.04-10.85	0.69-7.19	0.02-4.44	0.10-0.53	0.18-1.55	0.67-2.16	0.00-1.26	0.24-3.74	0.05-0.94	0.20-6.11	0.14-0.76	7.63-29.08
Mean	24.73	5.48	4.59	0.78	0.32	0.98	1.32	0.53	1.60	0.38	2.32	0.40	18.64
S.D.	2.99	2.77	2.03	1.12	0.14	0.39	0.47	0.38	1.18	0.30	2.07	0.25	7.33

Table 13c

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL MALES (mg FREE STEROID/24 HOUR VOLUME)

GLUCURONIDES										SULPHATES				TOTAL
No.	Age	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI		
19	30	8.79	4.69	1.82	0.26	0.69	2.80	0.64	1.71	0.23	3.05	0.50	25.18	
20	30	4.79	4.85	0.23	0.31	1.51	1.79	1.54	0.47	0.24	0.29	0.11	16.13	
21	31	3.43	1.37	0.25	0.18	0.54	1.01	0.00	0.65	0.06	0.48	0.18	8.15	
22	31	10.10	3.60	0.22	0.41	0.88	2.73	0.00	1.09	0.16	0.42	0.16	19.77	
23	31	3.25	2.67	0.77	0.56	0.79	0.98	0.48	1.08	0.40	3.23	0.25	14.46	
24	33	4.51	3.68	1.04	0.72	0.51	1.63	0.06	2.08	0.68	2.03	0.74	17.68	
25	34	7.32	5.66	0.98	0.08	0.45	1.97	0.56	1.84	0.25	4.15	0.82	24.08	
26	36	5.70	4.05	0.14	0.32	0.67	0.82	0.21	0.60	0.09	0.19	0.11	12.90	
27	38	5.26	3.24	0.54	0.82	0.91	0.65	0.06	0.64	0.18	1.46	0.23	13.99	
28	39	0.11	0.47	0.96	0.57	0.00	0.00	0.00	0.35	0.35	0.25	0.29	3.35	
Range	30-39	0.11-10.10	0.47-5.66	0.14-1.82	0.08-0.82	0.00-1.51	0.00-2.80	0.00-1.54	0.35-2.08	0.06-0.68	0.19-0.82	0.11-0.82	3.35-25.18	
Mean	33.30	5.32	3.43	0.70	0.42	0.70	1.44	0.36	1.05	0.26	1.56	0.34	15.57	
S.D.	3.33	2.89	1.59	0.53	0.24	0.39	0.91	0.48	0.62	0.18	1.48	0.26	6.69	

Table 13d

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL MALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	A	E	GLUCURONIDES						SULPHATES				TOTAL
				DHA + EPI	11-OA	11-OE	11-OHA	11-OHE		A	E	DHA	EPI	
29	40	1.06	1.49	0.07	0.06	0.75	0.59	0.98		0.15	0.04	0.10	0.04	5.33
30	40	3.70	2.20	0.08	0.37	1.03	2.45	1.10		0.45	0.14	0.35	0.09	12.00
31	40	1.61	1.30	0.20	0.21	0.32	0.34	0.42		0.12	0.06	1.07	0.07	5.72
32	41	1.63	1.53	0.31	0.12	0.53	0.69	0.12		0.28	0.08	2.10	0.11	7.50
33	42	2.12	0.90	0.23	0.23	0.41	0.76	0.30		0.85	0.19	2.27	0.24	9.01
34	44	1.26	2.02	0.40	0.07	0.25	0.31	0.02		0.38	0.23	0.68	0.05	5.67
35	48	0.11	0.51	0.81	0.85	0.00	0.16	0.38		0.35	0.36	0.26	0.29	4.08
36	48	1.48	0.38	0.06	0.09	0.18	0.46	0.19		0.05	0.03	0.09	0.02	3.03
Range	40-49	0.11-3.70	0.38-2.20	0.06-0.81	0.06-0.85	0.00-1.03	0.16-2.45	0.02-1.10		0.05-0.85	0.03-0.36	0.09-2.27	0.02-0.29	3.03-12.00
Mean	42.88	1.62	1.29	0.27	0.25	0.43	0.72	0.44		0.33	0.14	0.87	0.11	6.54
S.D.	3.44	1.02	0.66	0.25	0.26	0.33	0.73	0.39		0.25	0.11	0.88	0.10	2.88

Table 13a

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL MALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	GLUCURONIDES								SULPHATES				TOTAL
		A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI		
37	58	0.96	0.41	0.08	0.09	0.23	1.23	0.55	0.60	0.16	0.44	0.28	5.03	
38	63	0.22	0.41	0.08	0.06	0.10	0.22	0.00	0.08	0.06	0.02	0.09	1.30	
Range	58-63	0.22-0.96	0.41-0.41	0.08-0.08	0.00-0.09	0.10-0.23	0.22-1.23	0.00-0.55	0.08-0.60	0.06-0.16	0.02-0.44	0.09-0.28	1.30-5.30	
Mean	60.50	0.59	0.41	0.08	0.05	0.17	0.73	0.28	0.34	0.11	0.23	0.19	3.17	
S.D.	5.54	0.52	0.00	0.00	0.06	0.09	0.71	0.39	0.37	0.07	0.30	0.13	2.64	
Overall Range	17-63	0.11-10.85	0.38-7.19	0.02-4.44	0.00-0.85	0.00-1.55	0.00-2.80	0.00-1.54	0.05-3.74	0.03-0.94	0.09-6.11	0.02-0.82	1.30-29.08	
Overall Mean	32.16	4.23	3.25	0.58	0.32	0.73	1.18	0.44	1.11	0.28	1.68	0.31	14.09	
S.D.	10.77	2.88	2.06	0.79	0.21	0.42	0.70	0.40	0.96	0.23	1.70	0.23	7.81	

As far as the author was able to ascertain, none of the normal women studied were using steroidal contraceptives. The values for the individual urinary 17-OS sulphate and glucuronide levels of the individual females, and the means and standard deviations of the groups, are presented in Tables 14(a-e). From these tables, the very wide range of values in both sexes for all the steroids studied, especially for DHAS and DHAG plus EPIG, can be observed. The overall coefficient of variation for DHA in both fractions in both sexes varies from 89% to 136%. These wide variations emphasise the difficulty encountered in obtaining suitable control data. To simplify the situation, any steroid level which was above the upper limit of the seventy-five normal individuals investigated was considered to be abnormal. Similar wide range values for the individual 17-OS, without distinguishing between the contribution made by sulphate and glucuronide fractions, have been reported by Keutman and Mason (1967). Despite this wide variation in excretion of the 17-OS by both sexes, males excrete significantly much greater amounts of each individual 17-OS than females, with the exception of aetiocholanolone sulphate and 11-hydroxyaetiocholanolone glucuronide where the mean values for males and females were very similar (Table 15). These higher excretion values are to be expected, since it is well known that total urinary 17-OS levels are much higher in males than females. Furthermore, the main precursors of the urinary

Table 14a

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL FEMALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	GLUCURONIDES						SULPHATES				TOTAL	
		A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA		EPI
1	18	1.02	1.53	0.12	0.12	0.29	0.51	0.34	0.19	0.40	0.39	0.09	5.00
2	19	0.50	0.71	0.07	0.00	0.36	0.34	0.14	0.04	0.00	0.14	0.03	2.33
3	19	2.65	0.95	0.26	0.21	0.89	1.01	0.52	0.31	0.28	0.23	0.16	7.37
Range	18-19	0.50- 2.65	0.71- 1.53	0.07- 0.26	0.00- 0.21	0.29- 0.89	0.34- 1.01	0.14- 0.52	0.04- 0.31	0.00- 0.40	0.14- 0.39	0.03- 0.16	2.33- 7.37
Mean	18.67	1.39	1.06	0.15	0.11	0.51	0.62	0.33	0.18	0.19	0.25	0.09	4.90
S.D.	0.58	1.12	0.42	0.10	0.11	0.33	0.35	0.19	0.14	0.20	0.13	0.07	2.52

Table 14b

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL FEMALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	A	E	GLUCURONIDES					SULPHATES				TOTAL
				DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI	
4	20	4.43	2.27	0.03	0.10	0.23	0.57	0.34	0.67	0.53	0.78	0.12	10.07
5	20	3.24	2.77	0.02	0.25	0.26	0.71	0.26	0.70	0.27	0.59	0.32	9.39
6	20	3.10	2.21	0.15	0.19	0.73	0.96	0.29	0.69	0.36	0.61	0.29	9.58
7	21	1.76	1.46	0.04	0.09	0.40	0.66	0.29	0.60	0.14	0.44	0.12	6.00
8	21	3.28	1.78	0.07	0.25	0.90	1.08	0.00	0.63	0.07	0.48	0.14	8.68
9	23	2.78	4.00	0.08	0.17	0.46	0.29	0.41	0.22	0.06	0.00	0.00	8.47
10	25	1.51	2.22	0.02	0.07	0.49	0.29	0.15	0.39	0.24	0.18	0.11	5.67
11	25	2.22	1.95	0.41	0.22	0.73	0.52	0.49	0.78	0.55	0.72	0.13	8.72
12	26	2.63	2.27	0.06	0.21	0.44	0.72	0.44	0.17	0.24	0.24	0.04	7.46
13	26	3.21	3.25	0.47	0.24	1.54	0.63	1.00	0.03	0.16	0.24	0.06	10.83
14	26	3.59	2.24	0.25	0.24	0.77	0.91	0.70	0.00	0.16	0.04	0.07	8.97
15	28	2.70	1.71	0.00	0.27	0.15	1.10	0.30	0.89	0.22	0.58	0.10	8.02
16	29	1.61	1.92	0.22	0.17	0.68	1.38	0.32	0.03	0.03	0.14	0.16	6.66
Range	20-29	1.51-4.43	1.46-2.77	0.00-0.47	0.07-0.27	0.15-1.54	0.29-1.38	0.00-1.00	0.00-0.89	0.03-0.55	0.00-0.78	0.00-0.32	5.67-10.83
Mean	23.85	2.77	2.31	0.14	0.19	0.60	0.76	0.38	0.45	0.23	0.39	0.13	8.35
S.D.	3.18	0.84	0.68	0.16	0.07	0.36	0.32	0.25	0.32	0.16	0.26	0.09	1.55

Table 14c

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL FEMALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	A	E	GLUCURONIDES						SULPHATES				TOTAL
				DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI		
17	30	1.27	1.13	0.03	0.11	0.31	0.99	0.61	0.21	0.19	0.01	0.09	4.95	
18	31	0.94	2.25	0.05	0.14	0.34	0.60	0.35	0.49	0.16	0.72	0.08	6.12	
19	31	0.16	0.49	0.07	0.00	0.26	0.48	0.00	0.02	0.06	0.04	0.11	1.68	
20	32	0.86	2.41	0.32	0.18	0.43	0.58	0.34	0.37	0.25	0.17	0.04	5.95	
21	32	1.24	1.16	0.20	0.09	1.04	1.28	0.75	0.33	0.35	0.14	0.06	6.64	
22	33	1.90	2.35	0.05	0.06	0.52	0.70	0.40	0.33	0.21	0.10	0.06	6.68	
23	34	0.53	0.94	0.05	0.27	0.49	0.78	0.00	0.29	0.28	0.15	0.06	3.84	
24	35	1.65	1.44	0.15	0.14	0.39	0.34	0.00	0.94	0.19	0.80	0.33	6.37	
25	37	0.64	0.43	0.06	0.08	0.29	1.38	0.24	0.45	0.38	0.69	0.26	4.90	
26	37	1.65	1.86	0.00	0.00	0.59	0.94	0.52	0.96	0.22	0.32	0.32	7.49	
27	39	0.29	0.30	0.04	0.20	0.20	0.24	0.26	0.17	0.09	0.50	0.06	2.36	
Range	30-39	0.16- 1.90	0.30- 2.41	0.00- 0.32	0.00- 0.27	0.20- 1.04	0.24- 1.38	0.00- 0.75	0.02- 0.96	0.06- 0.38	0.01- 0.80	0.04- 0.33	1.68- 7.49	
Mean	33.73	1.01	1.34	0.09	0.12	0.45	0.76	0.32	0.41	0.22	0.33	0.13	5.27	
S.D.	2.94	0.58	0.78	0.09	0.08	0.24	0.36	0.25	0.29	0.16	0.29	0.11	1.93	

Table 14d

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL FEMALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	A	E	GLUCURONIDES						SULPHATES				TOTAL
				DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	A	E	DHA	EPI		
28	40	0.92	1.06	0.11	0.12	0.53	0.50	0.29	0.03	0.12	0.12	0.01	3.80	
29	40	1.03	1.34	0.03	0.16	0.24	0.77	0.28	0.21	0.53	0.18	0.39	5.20	
30	40	0.52	0.49	0.05	0.19	0.71	0.88	0.45	0.07	0.03	0.00	0.00	3.39	
31	40	1.36	1.78	0.15	0.21	0.85	1.45	0.09	0.00	0.00	0.53	0.25	6.67	
32	42	1.12	1.70	0.07	0.09	0.31	0.52	0.19	0.37	0.42	0.14	0.07	5.00	
33	42	0.31	0.35	0.05	0.06	0.25	0.39	0.16	0.07	0.03	0.04	0.03	1.74	
34	43	1.41	1.51	0.22	0.02	0.16	0.23	0.05	0.20	0.16	0.38	0.12	4.46	
35	45	1.36	1.24	0.25	0.21	0.67	0.69	0.31	0.30	0.21	0.75	0.11	6.10	
36	46	0.11	0.28	0.04	0.03	0.04	0.03	0.01	0.02	0.02	0.06	0.02	0.66	
37	49	0.31	0.74	0.00	0.00	0.19	0.15	0.11	0.24	0.20	0.22	0.01	2.17	
Range	40-49	0.11-1.41	0.28-1.78	0.00-0.25	0.00-0.21	0.04-0.85	0.03-1.45	0.01-0.45	0.00-0.37	0.00-0.53	0.00-0.75	0.00-0.39	0.66-6.67	
Mean	42.70	0.85	1.05	0.10	0.11	0.40	0.56	0.19	0.15	0.17	0.24	0.10	3.92	
S.D.	3.09	0.49	0.56	0.08	0.08	0.27	0.41	0.14	0.13	0.18	0.24	0.13	1.95	

Table 14e

EXCRETION OF 17-OXOSTEROID GLUCURONIDES AND SULPHATES BY NORMAL FEMALES (mg FREE STEROID/24 HOUR VOLUME)

No.	Age	<u>GLUCURONIDES</u>						<u>SULPHATES</u>				<u>TOTAL</u>	
		A	E	DHA + EPI	11-DA	11-OE	11-OHA	11-OHE	A	E	DHA		EPI
Overall Range	18-49	0.11- 4.43	0.28- 4.00	0.00- 0.47	0.00- 0.27	0.04- 1.54	0.03- 1.45	0.00- 1.00	0.00- 0.96	0.00- 0.55	0.00- 0.80	0.00- 0.39	0.66- 10.83
Overall Mean	31.46	1.62	1.58	0.11	0.14	0.49	0.69	0.31	0.34	0.21	0.28	0.12	5.96
S.D.	8.91	1.11	0.85	0.12	0.08	0.30	0.36	0.23	0.28	0.15	0.25	0.10	2.57

Table 15

COMPARISON OF THE INDIVIDUAL 17-OS URINARY LEVELS OF

NORMAL MALES AND FEMALES

Steroid	Males (38)* Mean \pm S.D.	Females (37)* Mean \pm S.D.	p Value
AS	1.11 \pm 0.96	0.34 \pm 0.28	<0.001
ES	0.28 \pm 0.23	0.21 \pm 0.15	not significant
DHAS	1.68 \pm 1.70	0.28 \pm 0.25	<0.001
EPIS	0.31 \pm 0.23	0.12 \pm 0.10	<0.001
AG	4.23 \pm 2.88	1.62 \pm 1.11	<0.001
EG	3.25 \pm 2.06	1.58 \pm 0.85	<0.001
DHAG + EPIG	0.58 \pm 0.79	0.11 \pm 0.12	<0.001
11-OAG	0.32 \pm 0.21	0.14 \pm 0.08	<0.001
11-OEG	0.73 \pm 0.43	0.49 \pm 0.30	<0.01
11-OHAG	1.18 \pm 0.70	0.69 \pm 0.36	<0.001
11-OHEG	0.44 \pm 0.40	0.31 \pm 0.23	not significant

* No. of subjects in each group

17-OS, DHAS, DHA, testosterone and androstenedione, excluding androstenedione, are secreted in larger amounts by males compared to females (MacDonald et al., 1965).

Figures 24 and 25 demonstrate that the period for maximum excretion of the individual 17-OS for both sexes occurs in the 20 - 29 age group. This is in agreement with the results obtained by Hamburger (1948) for maximum excretion of the total urinary 17-OS.

The greater of the two urine fractions is undoubtedly the glucuronide fraction, consisting of approximately 76% of the total urinary 17-OS in males and 83% in females. A similar pattern is observed with the 11-oxygenated 17-OS. In males they make up 19% of the total urinary 17-OS but in females this rises to 28% of the total. This group of $C_{19}O_3$ (i.e. 11-oxygenated) steroid glucuronides consists of approximately equal amounts of 11-oxygenated androsterone and 11-oxygenated aetiocholanolone in both sexes, with 11-OE and 11-OHA quantitatively the most prominent (Fig. 26a, b). These graphs of the urinary excretion of the $C_{19}O_3$ steroid glucuronides in different age groups indicate that the peak of 11-oxygenated androsterone excretion in males occurs in the 30 - 40 age range, whereas the peak of 11-oxygenated aetiocholanolone excretion occurs in the 20 - 30 age range, with 11-OHE showing a second peak in the 40 - 50 age range. In contrast to this, the excretion patterns of the $C_{19}O_3$ steroids in women were much more uniform with all four steroids

Urinary excretion of individual 17-oxosteroids in normal males

Fig. 24a

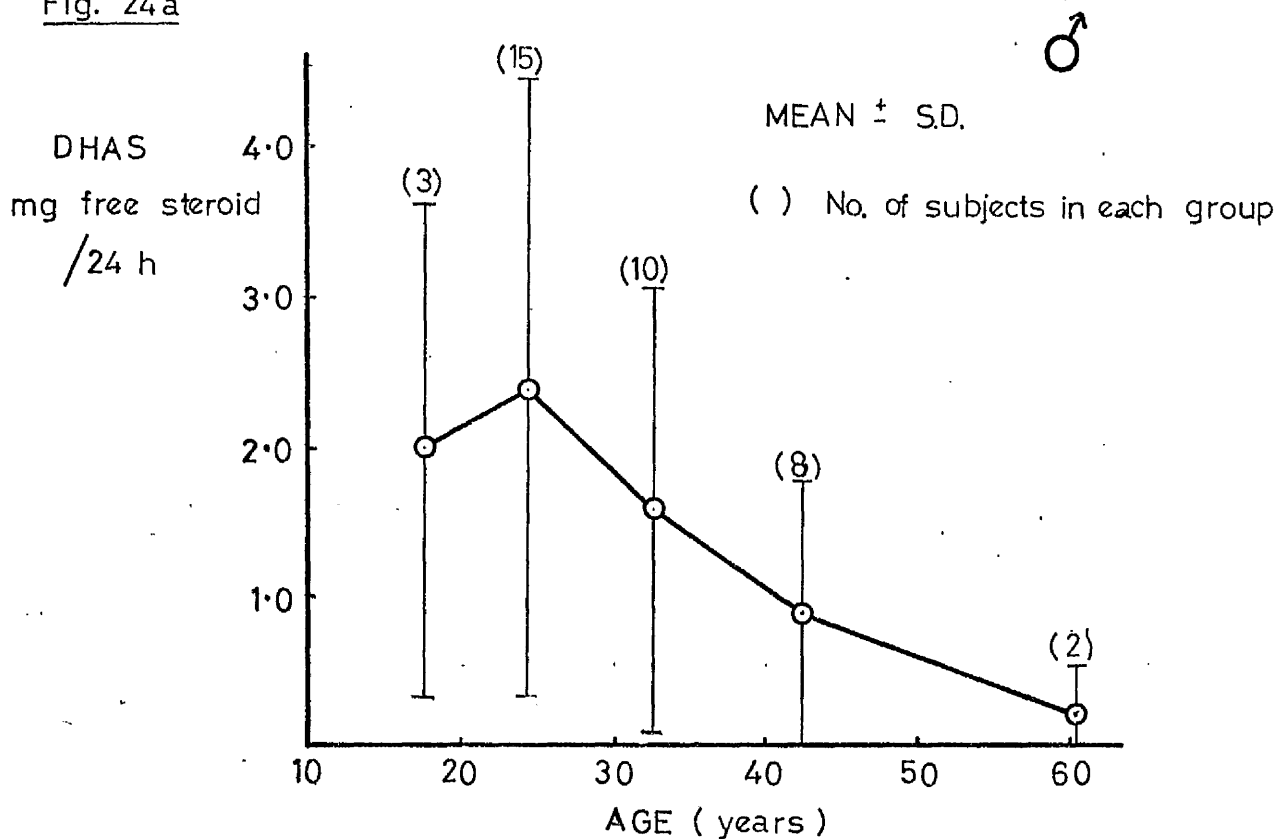
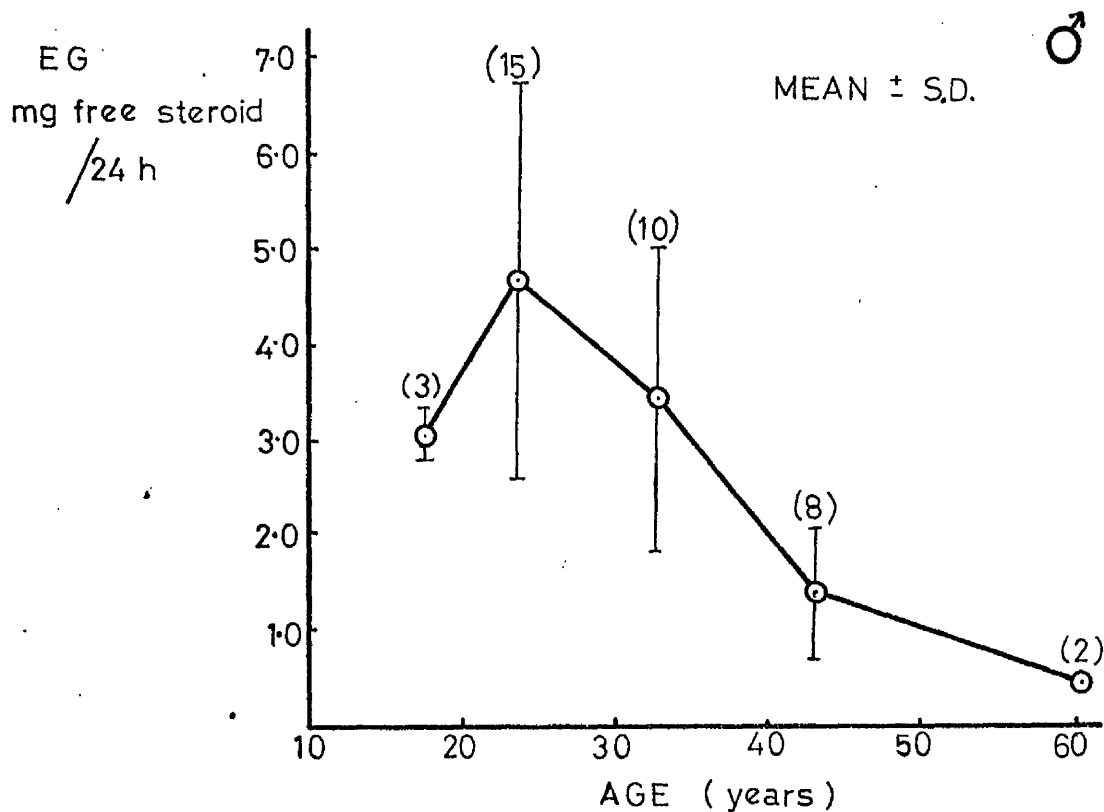


Fig. 24b



EG - Aetiocholanolone Glucuronide

Urinary excretion of individual 17-oxosteroids in normal females

Fig. 25a

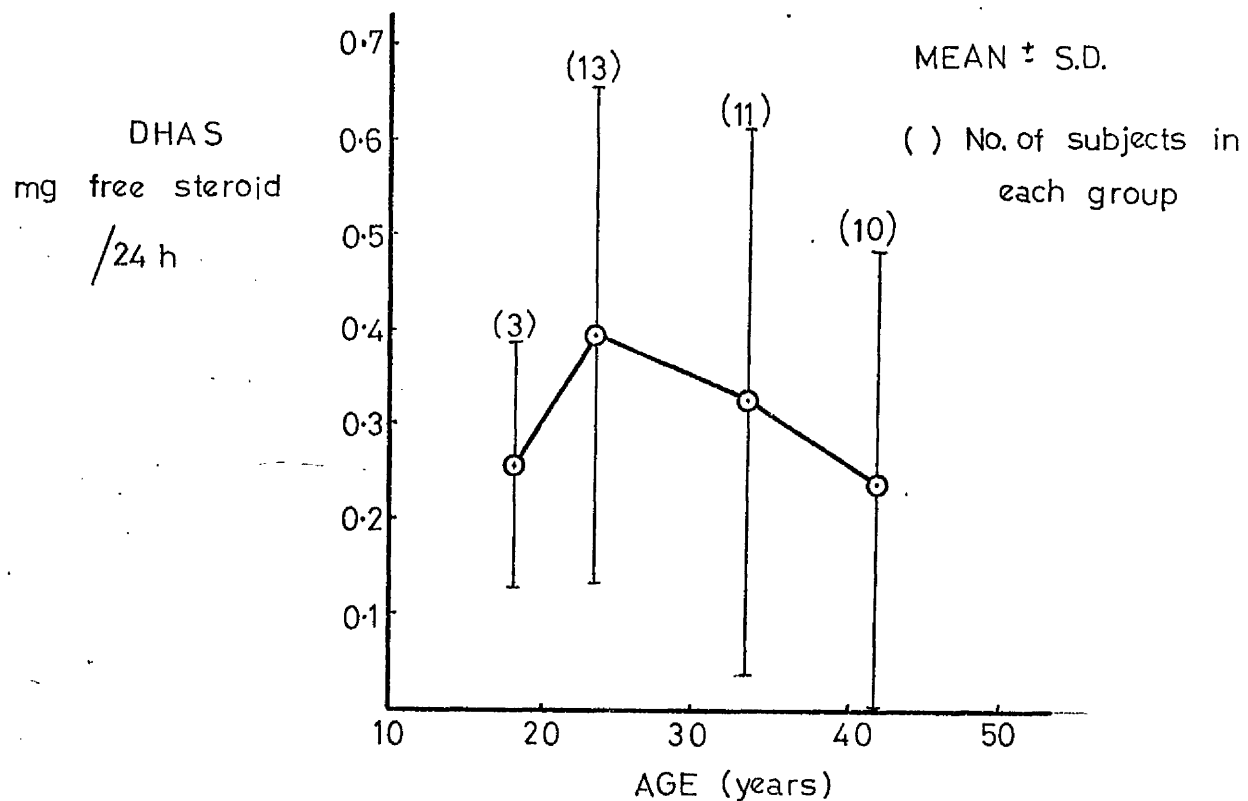
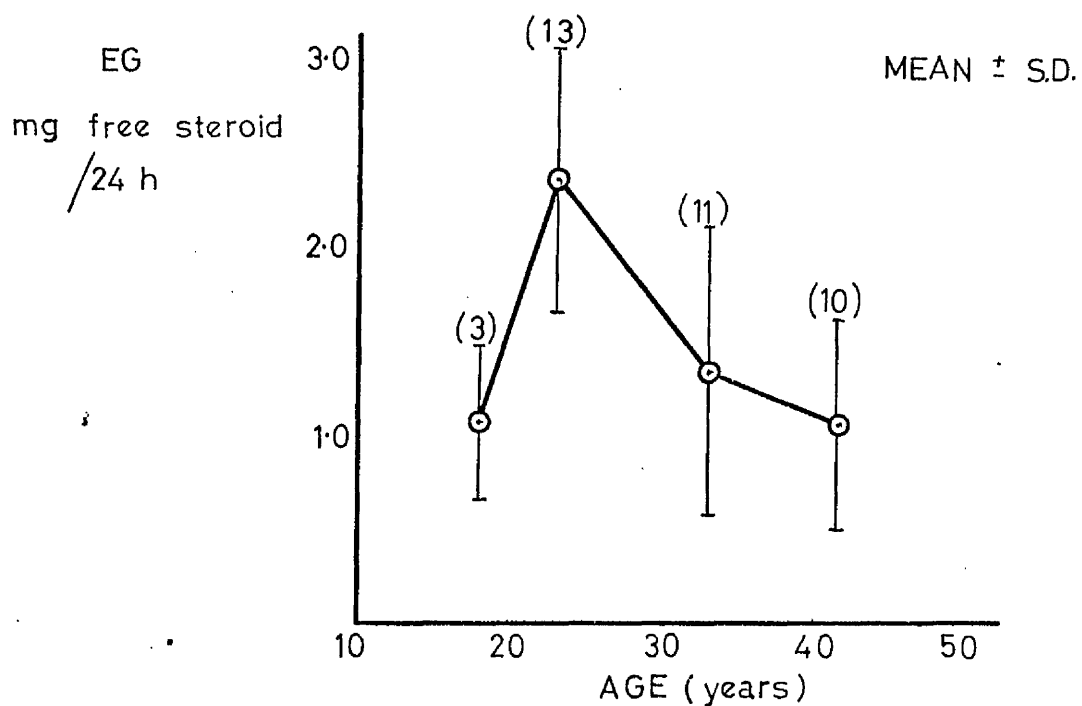


Fig. 25b



EG - Aetiocholanolone Glucuronide

Legend for figure 26

Mean urinary excretion levels of $C_{19}O_3$ steroid
(i.e. 11-oxygenated-17-oxosteroid) glucuronides in
different age groups in normal males (26a)
and females (26b).



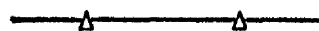
= 11- OXOANDROSTERONE



= 11- OXOAETIOCHOLANOLONE



= 11- HYDROXYANDROSTERONE



= 11- HYDROXYAETIOCHOLANOLONE

Urinary excretion of 11-oxygenated-17-oxosteroids in normal subjects

Fig. 26a

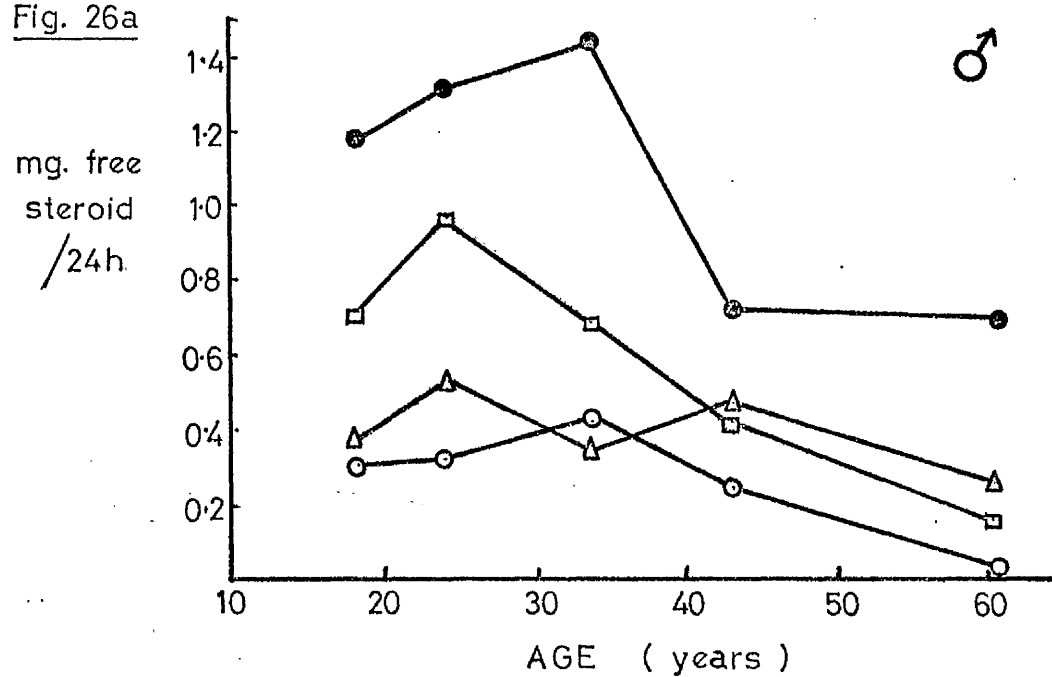
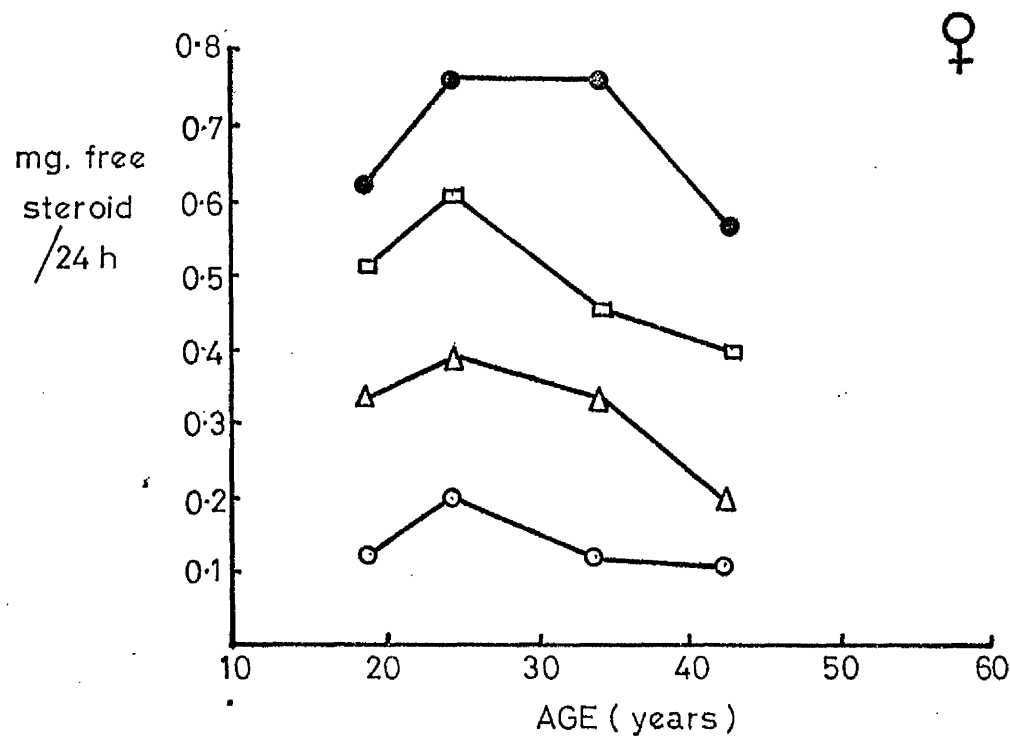


Fig. 26b



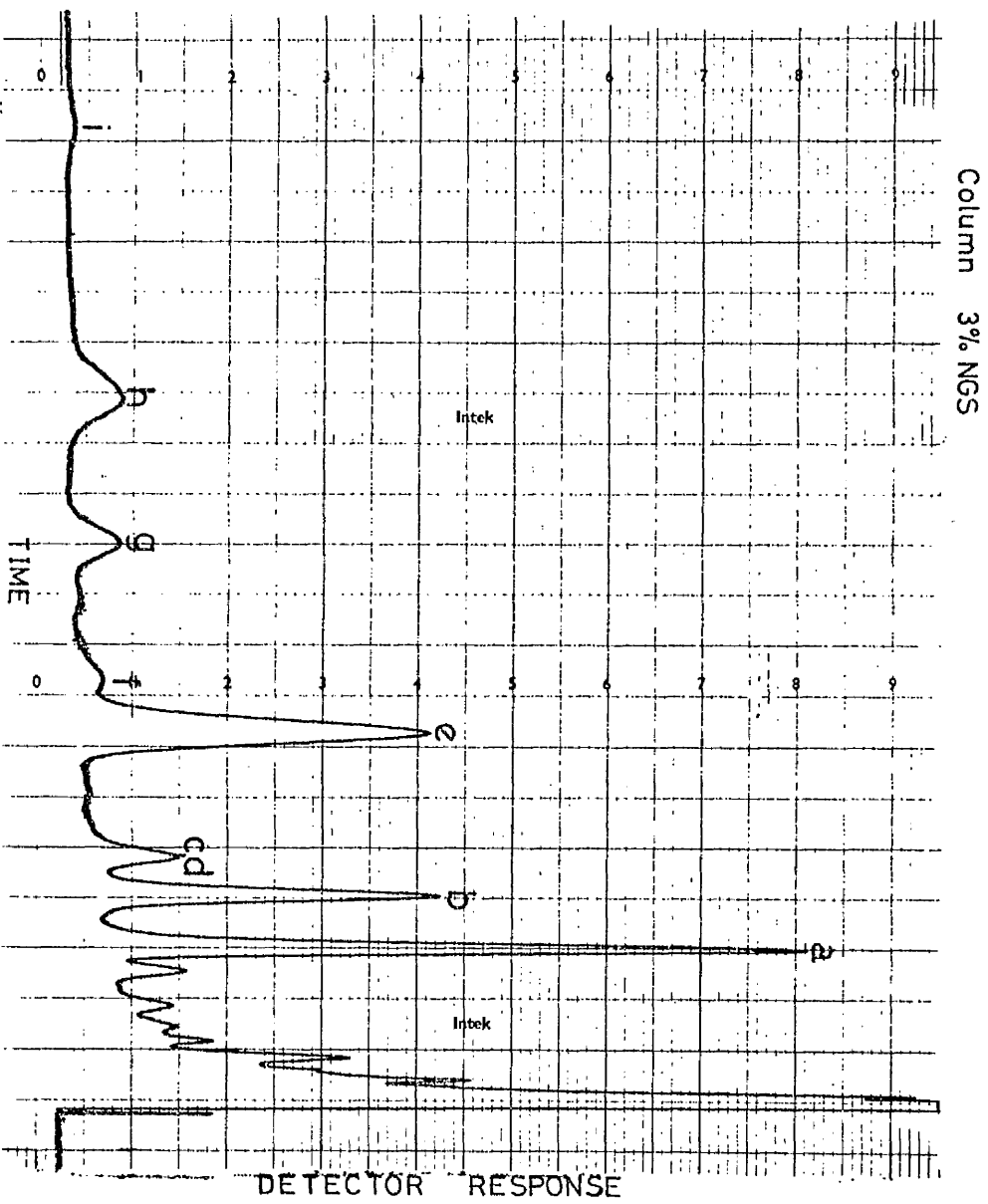
showing maximum excretory levels in the 20 - 30 age range, although it must be noted that the level for 11-OHA remained at this maximum into the 30 - 40 age group. Of the whole glucuronide fraction, androsterone and aetiocholanolone were quantitatively the most important in both males and females. In fact, these two steroids together constitute the major portion of the total urinary 17-OS in normal subjects. Of these two steroids, the more prominent in 71% of the males was androsterone glucuronide, and aetiocholanolone glucuronide in 57% of the females. The typical steroid glucuronide excretion patterns in males ($A > E > DHA$) and in females ($E > A > DHA$) are illustrated by the gas chromatographic tracings shown in Figs. 27 and 28.

Of the sulphate fraction, DHAS was quantitatively the most important in 53% of the males, but only in 32% of the females. In 49% of the females, AS was quantitatively the most important, and in 14% of the normal females ES was the most important. The females showed no typical steroid sulphate excretion pattern, other than that EPIS was the minor steroid sulphate. The typical urinary 17-OS sulphate excretion pattern for males was $DHA > A > EPI > E$, and is illustrated in the gas chromatographic tracing in Fig. 29.

In all cases, the total urinary 17-OS excretion (i.e. the sulphate plus glucuronide fraction) for males was much higher than that for females in the respective age groups (Table 16). The overall mean excretion for the thirty-eight males (mean age 32) was 14.09 mg/24 h, whereas the mean for the females

Figure 27

GLC tracing of the 17-oxosteroid glucuronide fraction from the urine
of a normal male



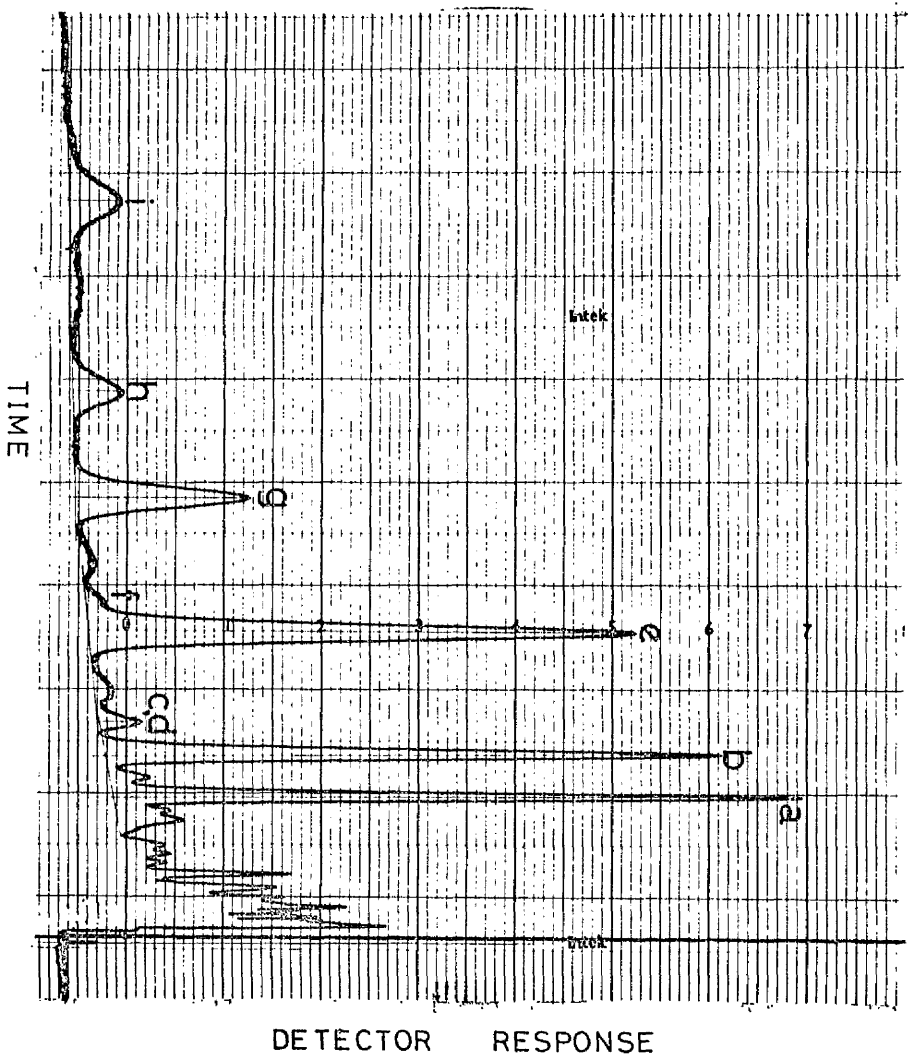
Typical male steroid glucuronide excretion pattern with androsterone(a)

> aetiocholanolone(b) > DHA + epiandrosterone (c,d)

Figure 28

GLC tracing of the 17-oxosteroid glucuronide fraction from
the urine of a normal female

Column 3% NGS

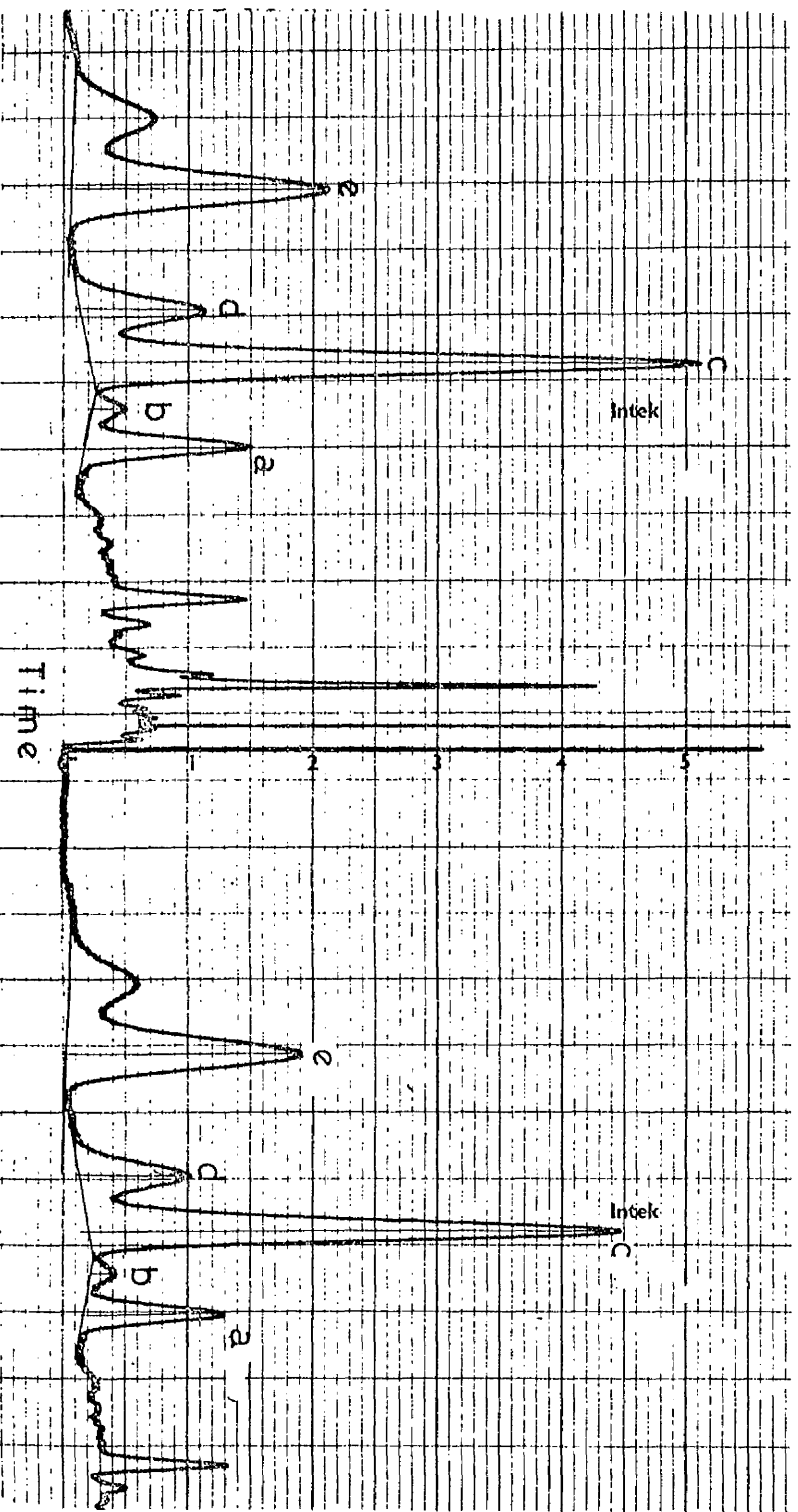


Typical female steroid glucuronide excretion pattern with
aetiocholanolone (b) > androsterone (a) > DHA + epiandrosterone (c, d)

Figure 29

GLC tracings of the 17-oxosteroid sulphate fraction from the
urine of a normal male

Column 3% QF-1 ; duplicate analyses using the automatic solid
injection system.



Typical male steroid sulphate excretion pattern with DHA (c) > androstosterone (a)
> androstosterone (d) > androstosterone (b).

Table 16

COMPARISON OF TOTAL URINARY 17-OS EXCRETION IN DIFFERENT

AGE GROUPS OF NORMAL MALES AND FEMALES

Age Group	Males Mean \pm S.D.	Females Mean \pm S.D.	p Value
17 - 19	13.88 \pm 2.08	4.90 \pm 2.52	< 0.01
20 - 29	18.64 \pm 7.33	8.35 \pm 1.55	< 0.001
30 - 39	15.57 \pm 6.69	5.27 \pm 1.93	< 0.001
40 - 49	6.54 \pm 2.88	3.92 \pm 1.95	< 0.025
Entire Group	14.09 \pm 7.81	5.96 \pm 2.57	< 0.001

(mean age 31.5) was 5.96 mg/24 h. In the present report the ratio of the total amount of aetiocholanolone (sulphate plus glucuronide) to the total amount of androsterone (sulphate plus glucuronide) was significantly greater ($p < 0.005$) for females than for males (Table 17). The values for the E/A ratios in this investigation are in agreement with those obtained by other investigators using other methods, following the same pattern, with the ratio for females being greater than that for males (Table 18). This ratio between the amount of aetiocholanolone to the amount of androsterone produced is partly under the influence of enzymes concerned with the reduction of the unsaturation involving the C-5 atom of the 17-OS precursors and partly a reflection of the relative amounts of the various 17-OS precursors. Both DHA and testosterone are converted to androstenedione, which is converted into equal portions of A and E by Δ^4 -reductase located in the liver parenchymal cells (Baulieu and Mauvais-Jarvis, 1964a,b). The Δ^4 -5 α -reductase is located in the endoplasmic reticulum (Forchielli and Dorfman, 1956; McGuire and Tomkins, 1958) and the Δ^4 -5 β -reductase in the soluble cytoplasm of these liver cells (Tomkins, 1956). These enzymes both require NADPH as the hydrogen donor and are susceptible to various precursors and hormonal influences. Exogenous DHA suppresses Δ^4 -5 α -reductase and a rise in E develops, much as in malignancy of adrenals with excess DHA formation (Kirschner and Lipsett, 1964). Adlercreutz et al. (1972) reported that, following administration of DHAS in

Table 17

COMPARISON OF THE RATIOS OF E/A FOR MALES AND FEMALES

Age Group	Male E/A ratio	Female E/A ratio	p Value
	Mean \pm S.D.	Mean \pm S.D.	
17 - 19	0.66 \pm 0.03 (3)	1.11 \pm 0.62 (3)	not significant
20 - 29	0.73 \pm 0.27 (15)	0.84 \pm 0.29 (13)	< 0.10
30 - 39	0.74 \pm 0.42 (10)	1.31 \pm 0.74 (11)	< 0.05
40 - 49	0.91 \pm 0.55 (8)	1.33 \pm 0.44 (10)	< 0.10
Entire Group	0.78 \pm 0.39 (38)	1.14 \pm 0.55 (37)	< 0.005

The number in parenthesis represents the no. of subjects in each group

Table 18

COMPARISON OF E/A RATIOS FROM DIFFERENT AUTHORS

	1	2	3	4	5
Male E/A ratio	0.78	0.82	0.97	0.70	0.75
Female E/A ratio	1.14	0.87	1.11	0.86	1.07

- 1) Present report: internal standard, alumina column chromatography and g.l.c.
- 2) Feher (1966): paper chromatography, Zimmermann reaction.
- 3) Keutmann and Mason (1967): internal standard, g.l.c.
- 4) Rivera et al. (1967): silica gel chromatography, g.l.c.
- 5) Vestergaard (1962): column chromatography, Zimmermann reaction.

patients with hypercholesterolaemia or hyperlipaemia (these patients tend to have low plasma and urine levels of DHAS and other 17-OS sulphates), normal mean levels of plasma 17-OS sulphates and normal mean urinary DHA and androsterone excretion were reached, but the urinary excretion of aetiocholanolone increased to a value 2 - 3 times as high as those seen in both young and older age groups. Contrary to this, Johnsen (1968) demonstrated a significant decrease in the E/A ratio of 11 out of 12 normal subjects after administration of large doses of testosterone. The mean reported secretion rate for testosterone in females is 0.14 mg/day and in males 7.0 mg/day (Baulieu and Mauvais-Jarvis, 1964b; MacDonald et al., 1965; Horton and Tait, 1966). Thus, the difference in the secretion rates of testosterone for males and females might help explain the difference in the E/A ratio shown by males and females. Further evidence to support this theory is given by Johnsen (1968) in his study of castrated males and males with testicular insufficiency. He reported that the E/A ratio of this group was substantially higher than that for normal men. Similarly, Keutman and Mason (1967) reported that the E/A ratio of two castrated males was similar to that of normal females.

The contribution of the C₂₁ steroids to the E/A ratio, although probably quite small, must be taken into consideration. The side chain of the 11-deoxy C₂₁ steroids has been shown to strongly favour reduction of the Δ^4 double-bond to give 5 β steroids, thus metabolism of 11-deoxy C₂₁ steroids to urinary

17-OS will elevate the E/A ratio. Those C₂₁ steroids with an oxygen atom at the C-11 position have been shown to favour 5 β and 5 α orientation respectively, depending on whether this atom is present as a ketone or hydroxyl (Slaunwhite et al., 1964). However, these C₂₁ steroids with functional groups at C-11 retain them on metabolism to 17-OS, and are excreted as 11-OHA, 11-OHE, 11-OA and 11-OE. Therefore, they have no effect on the E/A ratio. This ratio is also affected by various chemicals; for example, triiodothyronine stimulates Δ^4 -5 α -reductase resulting in an increase in A (Kirschner et al., 1963). Thus, it can be seen that the E/A excretion ratio does not result from a straightforward conversion of androstenedione into equal amounts of A and E, but from a very highly complex origin with many factors influencing the final outcome.

In this present investigation, the effect of the menstrual cycle on the urinary excretion of 17-OS sulphates was not studied. There have been numerous publications on the relationship of the urinary excretion of these steroids to the menstrual cycle (see Loraine and Bell, 1971). For the most part, these studies have indicated that the excretion of 17-oxosteroids does not undergo any fluctuations which might be related to the different phases of the menstrual cycle. Determination of the 17-oxosteroids in these previous publications has mainly been carried out with rather unspecific colorimetric methods. Using specific gas chromatographic techniques, however, Adlercreutz et al. (1967) were able to

show that the amounts of oestrone and oestradiol in the urine were significantly correlated with those of androsterone and aetiocholanolone during the menstrual cycle. In addition, these investigators found a significant correlation between the excretion of DHA and 5β -pregnane- $3\alpha,20\alpha$ -diol during the menstrual cycle. Using similar specific gas chromatographic techniques, Jänne (1971) reported that statistical analyses revealed a significant difference between the follicular (F) and the luteal (L) phases of the menstrual cycle in the excretion of epiandrosterone ($L > F$, $p < 0.05$) and DHA sulphates ($L > F$, $p < 0.05$). The total urinary excretion of the 17-OS sulphates was also significantly ($P < 0.05$) raised during the post-ovulatory phase of the menstrual cycle. The mean increase in the excretion of DHAS during the luteal phase of the menstrual cycle was 314 ± 207 (S.D.) $\mu\text{g}/24 \text{ h}$, and that of total 17-OS sulphates $617 \pm 158 \mu\text{g}/24 \text{ h}$. The mean excretions of androsterone and aetiocholanolone sulphates were slightly, but not significantly, greater during the post-ovulatory phase of the menstrual cycle. Although these increases in the excretion of DHAS and EPIS during the luteal phase are statistically significant, they are not great enough to affect the results from the control group of thirty-seven normal women studied in this investigation.

In the following investigation into the urinary excretion of 17-OS sulphates and glucuronides by patients suffering from different types of porphyria, the results

obtained from the seventy-five control subjects were used to set the higher limit for normal urinary excretion levels of these steroids. Levels above this upper limit were regarded as abnormally high.

SECTION VI

THE PLASMA 17-OXOSTEROID SULPHATES

A. INTRODUCTION.

These forementioned investigations established the occurrence of irregularities in the urinary excretion of 17-oxosteroids in certain patients with hepatic porphyria, and it was these considerations which motivated the current studies concerning the characterization and quantitation of conjugated steroids in human peripheral plasma. Considerable data have been accumulated on the nature of steroids found in human blood and plasma. Migeon and Plager (1954a,b) reported the identification and isolation of DHA in human plasma. This was followed by the isolation and identification of androsterone (Clayton et al., 1955; Migeon, 1956). These steroids were shown to be present in blood plasma in conjugated form, and the fact that these conjugates were hydrolyzed by continuous ether extraction at pH 0.8 suggested them to be sulphate conjugates. The finding that the conjugates of DHA and androsterone in blood were cleaved by solvolysis, described by Burstein and Lieberman (1958), further suggested conjugation with sulphuric acid. Baulieu (1960) reported evidence for the presence of aetiocholanolone in the blood of normal subjects in addition to DHA and androsterone. In this same paper, chromatographic evidence was presented showing that these three 17-oxosteroids were sulphate conjugated. The presence in blood of 17-oxosteroids in forms other than solvolyzable or sulphate conjugates, has also been studied and it is generally agreed that the amount of 17-oxosteroids in the free form is very low; less than 2 $\mu\text{g}/100$ ml plasma (Kellie and Smith, 1957;

Cohn et al., 1961; Wieland et al., 1965; Kirschner et al., 1965). The concentrations of glucuronides of 17-oxosteroids are also reported to be relatively low (Kellie and Smith, 1957; Migeon, 1960; Conrad et al., 1961; de Neve and Vermeulen, 1965; Baulieu, 1965). Thus, the bulk of the 17-oxosteroids in the plasma consists of sulphate conjugates, and the present investigation was directed to the study of these 17-oxosteroid sulphates in human plasma. A gas chromatographic method based on that described by Wang et al. (1968) was developed for the quantitative determination of the sulphate conjugates of androsterone, aetiocholanolone, DHA and epi-androsterone in plasma.

B. MATERIALS AND METHOD FOR THE DETERMINATION OF THE
17-OXOSTEROID SULPHATES IN PLASMA.

Materials.

Most of the chemical reagents, radioactive compounds and solvents have been described previously in the materials section of the urinary methods. Any additional chemical reagents used were BDH Analar grade reagents.

For this plasma method, the ethanol and methanol were supplied from BDH's Aristar range of very high purity chemicals.

The isopentane (2-methylbutane) was also a BDH solvent, specifically purified for chromatographic purposes.

The chloroform was obtained from Reeve Angel's range of CT solvents for chromatography.

Preparation of Chromatographic Columns.

Sephadex Columns. The Sephadex LH-20 columns provided a simple and non-destructive chromatographic procedure affording a considerable purification of the fraction of plasma extract containing the steroid monosulphates. The Sephadex LH-20 was supplied by A.B. Pharmacia, Uppsala, Sweden. This Sephadex LH-20 is a lipophilic derivative of ordinary Sephadex formed by introducing 2-hydroxy-ethyl groups in ether linkage to the free hydroxyl groups of the regular Sephadex. This makes the Sephadex LH-20 more polar, resulting in greater retardation

of polar compounds like the 17-oxosteroid sulphates. All the sulphate conjugated 17-oxosteroids, when chromatographed on Sephadex LH-20 in chloroform/methanol, 1:1, containing 0.01M sodium chloride, had elution volumes larger than the total column volume. When this solvent system was used, the major serum lipids were eluted in the following order: phospholipids, triglycerides, cholesterol esters and cholesterol (Nystöm and Sjövall, 1965). The latter compound appeared in the eluate at approximately 10 - 20 ml. The steroid monosulphates analysed in this work were eluted between 25 - 50 ml, and the fraction used for this analysis was collected between 20 - 58 ml of effluent. The only major impurity in the fraction of steroid monosulphates was part of the serum cholesterol. Further practical and theoretical aspects of the chromatographic behaviour of these sulphate conjugated steroids are discussed in a paper by Vihko (1966).

The chloroform/methanol mixture containing 0.01M sodium chloride was prepared by adding chloroform to a 0.02M solution of the electrolyte in methanol. Four grams of Sephadex LH-20 were allowed to swell in 25 ml of the forementioned solvent system for three hours before introduction into the glass columns. Each glass column consisted of a No. 2 sintered glass filter and a glass column with dimensions of 310 mm x 10 mm topped by a 150 ml reservoir. After washing the newly packed Sephadex with 100 ml of the solvent mixture, the columns were ready for use. The steroid monosulphates were collected in the 20 - 58 ml fraction, after the elution of the bulk of

the serum cholesterol. The free steroids emerged from the column somewhat earlier than cholesterol, partly associated with cholesterol peak, and thus were excluded from the fraction analysed. Analysis of the fraction representing 20 - 58 ml of the effluent, with omission of the solvolysis step, did not result in peaks corresponding to steroid peaks found after solvolysis of the fraction. In several studies, the fraction emerging earlier than 20 ml and the fraction between 58 - 100 ml of the effluent were solvolysed, chromatographed on the alumina columns and analysed by g.l.c. No peaks corresponding to those of the steroids in fractions between 20 - 58 ml of the effluent were seen in the chromatograms.

After each chromatographic run, the columns were eluted with 100 ml of the solvent mixture and re-used.

Alumina Columns. In addition to solvolysis and subsequent washing, a further purification step was needed to remove that part of the plasma cholesterol that was eluted with the steroid sulphates. Cholesterol sulphate which has been shown to be present in blood (Drayer and Lieberman, 1965; Moser et al., 1966) also emerged in the same fraction from the Sephadex LH-20 as the 17-oxosteroid sulphates. Chromatography on an alumina column was found to give the necessary purification.

The alumina, Woelm neutral activity Grade 1, was supplied by Camlab, Cambridge. The activity of the alumina was further lowered by addition of 8% by weight of distilled water. The column was prepared by loading 4 g of this weakened alumina in

benzene into the glass column which had previously been plugged with ethanol washed cotton wool. The top surface of the alumina was protected from disturbance by the addition of a thin layer of acid washed sand. The 17-oxosteroids were collected in 13 - 23 ml fraction of the effluent.

Cleansing of Glassware.

Special attention was paid to the cleaning of the glassware. All the glassware was washed with methanol, ethanol and water; then with detergent solution in an ultrasonic apparatus. It was finally rinsed with glass distilled water and ethanol.

Choice of Internal Standard.

The conditions which must be fulfilled before the choice of internal standard may be regarded as a suitable one have been discussed, with regard to the previous urinary methods. In this plasma procedure, pregnenolone sulphate appeared to fulfil all these requirements. It was eluted in the same fraction as the 17-oxosteroid sulphates from the Sephadex columns and in the same fraction as the 17-oxosteroids from the alumina columns; the ratios of peak heights obtained by g.l.c. for the control solutions and for the processed solutions were constant (Table 19); it was easily separable from the 17-oxosteroids by g.l.c. on a variety of columns and its peak was free of extraneous contamination. Its structure is very similar to DHA, which is the most labile of the 17-oxosteroids but, under the conditions employed, no significant

degree of breakdown was observed for any of the steroids investigated. On the question of whether there is any pregnenolone sulphate present in plasma or not, there is some disagreement. In 1965, Bégue reported the identification of pregnenolone sulphate in human peripheral plasma, but this was not supported by Vihko (1966) who used combined gas-liquid chromatography and mass spectrometry to identify the steroid sulphates in peripheral plasma. Using these same techniques, Ruokonen, Laatikainen, Laitinen and Vihko (1972) established the presence of free and sulphate-conjugated pregnenolone in human testis tissue. In this study, pregnenolone sulphate was not detected in any plasma samples from either sex. When pregnenolone sulphate was not added as an internal standard to plasma samples from both males and females, no peaks were formed on the gas chromatographic trace which would have contributed to the peak height of pregnenolone.

Method.

Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, model 3320.

Peripheral venous blood (5 - 10 ml) was withdrawn with a non-greased syringe, transferred to a lithium heparin bottle and centrifuged. The plasma was separated and stored at -20° until analysed.

A known amount of pregnenolone sulphate (5 μ g dissolved in 0.5 ml ethanol) was added as internal standard to each 2 ml aliquot of plasma.

Extraction of Plasma.

The plasma steroid sulphates were extracted twice with 4 ml ethanol, and the combined extracts were stored at -20° overnight. This induced further protein precipitation to occur. The combined extracts were then centrifuged, and the clear supernatant was evaporated to dryness in a water bath at $35 - 38^{\circ}$ under a stream of nitrogen.

Chromatography of Plasma Extract on Sephadex LH-20.

The dry plasma extract was taken up with 2 and 1 ml portions of 0.01M sodium chloride in chloroform/methanol, 1:1, centrifuged and the supernatants of the two portions pooled. The combined supernatants were carefully transferred on to the top of the Sephadex column with a pasteur pipette. After rinsing the top of the column with 1 ml of the same solvent system, the column was eluted with this solvent, and the effluent between 20 and 58 ml was collected in a round-bottomed flask and evaporated to dryness using the rota-evaporator.

Solvolysis.

The principles of Burstein and Lieberman (1958) were used in arriving at the following solvolysis procedure: Firstly, the round-bottomed flask containing the dried steroid sulphate fraction was rinsed out with 5 ml of sodium chloride saturated 0.1N sulphuric acid and then again with 10 ml of ethyl acetate. The two rinsings were combined, shaken for fifteen minutes,

transferred into separating funnels and separated. To the 5 ml of sodium chloride saturated sulphuric acid another 10 ml of ethyl acetate were added, and the procedure repeated. The two acidic ethyl acetate fractions were pooled and solvolysed at 39° overnight. After solvolysis, the ethyl acetate phase was washed with 5 ml portions of 20% sodium carbonate solution in water until alkaline, and then with 5 ml portions of water until neutral. The neutralized ethyl acetate was dried over anhydrous sodium sulphate and taken to dryness under a stream of nitrogen.

Alumina Column Chromatography.

A column with dimensions of 200 mm x 8 mm, and having a reservoir for solvent at the top, was closed with a plug of glass wool and filled partly with benzene. To this column 4 g of alumina weakened by 8% by weight of distilled water were added, followed by a thin layer of acid washed sand, and the solvent was allowed to flow until the top of the column was nearly dry. The dry steroid residue was dissolved in 0.5 ml and 2 x 2.5 ml portions of 1% ethanol/benzene, and added to the top of the column. The column was then washed with 8 ml of 1% ethanol/benzene to remove most of the cholesterol in the sample. Finally, the steroids were eluted with 10 ml of the 1% ethanol/benzene. This fraction was collected and taken to dryness under a stream of nitrogen.

Preparation of Trimethylsilyl Ethers(TMSE).

The trimethylsilyl ethers were prepared by the method of Thomas and Walton (1968). This procedure, described on page 84, was followed with the exception that only 0.2 ml of supernatant solution was added to the dry steroid residue for formation of the TMSE. After derivative formation, the trimethylsilyl ethers of the steroids were dissolved in hexane to give a concentration of pregnenolone internal standard of approximately 0.05 $\mu\text{g}/\mu\text{l}$. Along with the unknown plasma samples, residues containing equal amounts of 17-OS and pregnenolone were put through the preparation procedure, to check TMSE formation and correct peak height ratios.

Gas-Liquid Chromatography.

The conditions for gas-chromatographic analysis of the steroid trimethylsilyl ethers formed from purified plasma extract were the same as those applied to the analysis of the steroid trimethylsilyl ethers formed from the purified urine extract after sulphate solvolysis. These conditions have been reported in Section IV. The same glass column packed with 3% QF-1 was also used for the plasma analyses.

Calculation of Results.

Quantitation was achieved by comparing the ratios of the peak heights of pregnenolone to the other 17-OS, with standard solutions containing pregnenolone, androsterone, aetiocholanolone, DHA and epiandrosterone in the proportions 1:1:1:1:1, (see Fig. 30

for g.l.c. tracing of standard solution). Knowing the amount of pregnenolone added initially to the plasma, the following formula was used to calculate the number of μg of each steroid in a 100 ml of plasma:

$$\mu\text{g of steroid per 100 ml plasma} = \frac{a}{b} \times \frac{d}{c} \times \frac{e}{g} \times 100$$

Where:

a = peak height of 17-OS in the analytical specimen.

b = peak height of pregnenolone in the analytical specimen.

c = peak height of 17-OS in the standard.

d = peak height of pregnenolone in the standard.

e = number of μg of pregnenolone added initially to the plasma sample.

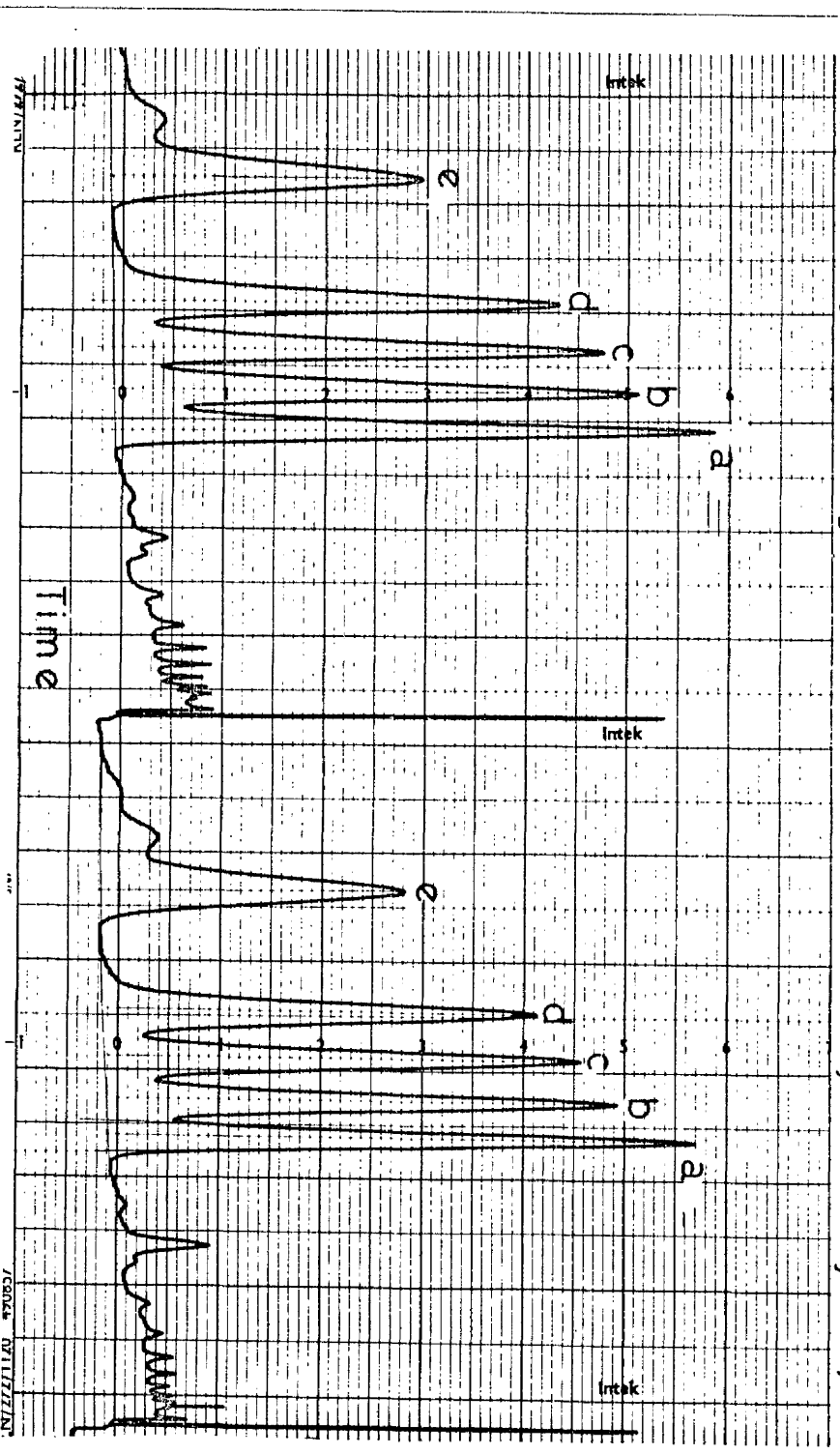
g = volume of plasma taken for analysis.

All the plasma values for the 17-oxosteroid sulphates in this thesis are expressed in terms of the concentration of free steroid per 100 ml plasma, unless otherwise stated.

Figure 30

GLC separation of the standard 17-oxosteroids used in the
plasma method

Duplicate analyses using the automatic solid injection system.



Column, 3 QF-1 : Temp 176°C : N₂ flow rate 50 ml/min

C. INVESTIGATION OF RELIABILITY OF THE METHOD FOR
DETERMINATION OF THE 17-OXOSTEROID SULPHATES IN PLASMA.

The quantitative determination of steroids in biological samples by gas-liquid chromatography depends upon the linear response of the detector, the stability of the derivative, the careful purification of the steroids prior to the final g.l.c. analysis and the accuracy of each step in the procedure.

Linearity of Detector.

The linearity of the detector response for all five steroids over a wide range of values has been demonstrated in Fig. 23. In accordance with the report of Grundy et al. (1965), the detector responses were directly proportional to the weights of the unsubstituted parent compounds. Thus, no correction factors were needed in the calculation of the g.l.c. results.

Stability of the TMSE derivatives.

The trimethylsilyl ethers were found to be stable for at least three days when stored in hexane solution in a stoppered test-tube.

Reliability of Procedure.

When an internal standard is introduced at the commencement of a method, it is necessary that the ratio of its amount to the amount of the steroids being measured remains constant throughout the analytical procedure. To establish whether

this was so, aliquots were taken from a solution containing the sulphate esters of androsterone, aetiocholanolone, DHA, epiandrosterone and pregnenolone. Some of these were solvolysed and used as controls, and the remainder were put through the steps of the method. The ratio of peak heights obtained by g.l.c. on the QF-1 column for the control solutions and for the processed solutions were compared, and the results are shown in Table 19. These data show that there was little change in the peak height ratios throughout the procedure.

Specificity.

The specificity of the method depends on the primary isolation on the Sephadex LH-20 column of the sulphate esters of pregnenolone, androsterone, aetiocholanolone, DHA and epiandrosterone and the subsequent separation of interfering substances from these solvolysed steroids by alumina column chromatography, partition in an ethanol-water-isopentane system and, finally, gas-liquid chromatography. Experiments with free radioactive steroids indicated that the unconjugated steroids were contained in the first 20 ml of effluent from the Sephadex column and not in the fraction containing the reference mono-sulphates. Analysis of the fraction representing 20 - 58 ml of the effluent with omission of the solvolysis step did not result in peaks corresponding to steroid peaks found after solvolysis of this fraction.

Evidence for the lack of interference from solvents and reagents was obtained by the analysis of a mixture of synthetic

Table 19

SUITABILITY OF PREGNENOLONE SULPHATE AS AN INTERNAL STANDARD

Procedure	Number of Experiments	Ratio	Ratio	Ratio	Ratio
		$\frac{A}{\text{Preg.}}$	$\frac{E}{\text{Preg.}}$	$\frac{\text{DHA}}{\text{Preg.}}$	$\frac{\text{EPI}}{\text{Preg.}}$
Control (Solvolysis)	5	1.894	1.727	1.606	1.495
	S.D.	0.060	0.097	0.053	0.055
	C.V.	3%	6%	3%	4%
Test (Solvolysis + Alumina Column Chromatography)	12	1.906	1.688	1.620	1.497
	S.D.	0.042	0.070	0.030	0.027
	C.V.	2%	4%	2%	2%
Test (Solvolysis + Alumina Column Chromatography + Partition)	6	1.911	1.691	1.582	1.441
	S.D.	0.058	0.095	0.050	0.048
	C.V.	3%	6%	3%	3%

S.D. - Standard Deviation

C.V. - Coefficient of Variation

steroid sulphates in ethanol (Table 20). The final g.l.c. analysis of this mixture after TMSE formation did not show peaks indicating any degradation of the steroid derivatives, or any interference in the steroid peaks. Further evidence to support this was provided by the fact that the recoveries of these synthetic steroids in relation to the recovery of pregnenolone sulphate, as calculated from these peak heights, were approximately 100% for all the steroids.

The plasma 17-OS TMSE derivatives were identified by their retention times relative to pregnenolone TMSE, which were identical to those of authentic standards (Table 21). When authentic steroids were added to plasma for recovery studies, no new peaks were formed; only the increases of the respective peak areas were observed. Similarly, if no internal standard was added to the plasma, no peaks were found on the gas chromatographic traces which would have contributed to the peak height of pregnenolone TMSE.

The shapes of the peak in both analytical and standard were identical, with no evidence of contamination from unknown peaks.

Finally, a batch of plasma samples from a pool was treated, as described, and analysed using both stationary phases, 3% QF-1 and 3% NGS. The data in Table 22 show that the differences between the results obtained when these two phases were used were trivial.

Table 20

ANALYSIS OF REFERENCE SYNTHETIC STEROID SULPHATES

Steroid Sulphate Added	μg Added	i.e. μg Free Steroid Added	n	μg Measured (Mean \pm S.D.)	Recovery %
AS	10	6.78	12	6.99 \pm 0.35	103%
ES	10	6.78	12	6.49 \pm 0.36	96%
DHAS	10	6.76	12	6.77 \pm 0.35	100%
EPIS	10	6.78	12	6.71 \pm 0.38	99%

n - number of determinations

S.D. - standard deviation

The steroid sulphates were dissolved in 4 ml ethanol and taken through the whole procedure. The final extracts were chromatographed on 3% QF-1, and the results expressed as amount of free steroid per aliquot.

Table 21

RETENTION TIMES OF 17-OS TMSE DERIVATIVES RELATIVE
TO PREGNENOLONE TMSE

Steroid	Authentic Steroids			Plasma Steroids		
	n	x	S.D.	n	x	S.D.
A	12	0.501	0.005	12	0.498	0.005
E	12	0.576	0.004	12	0.572	0.005
DHA	12	0.662	0.006	12	0.669	0.007
EPI	12	0.761	0.007	12	0.758	0.007

n - number of determinations

x - mean relative retention time

S.D. - standard deviation

The 3% QF-1 column was used for these analyses.

Table 22

ESTIMATION OF PLASMA 17-OS SULPHATES USING VARIOUS STATIONARY PHASES

Stationary Phase	n	A	E	DHA	EPI	
Pool A	3% QF-1	6	0.25 \pm 8%	-**	0.82 \pm 7%	0.11 \pm 9%
	3% NCS	6	0.22 \pm 9%	-**	0.95 \pm 6%*	
Pool B	3% QF-1	6	1.44 \pm 5%	0.62 \pm 5%	2.02 \pm 4%	1.26 \pm 7%
	3% NCS	6	1.51 \pm 6%	0.59 \pm 7%	3.30 \pm 4%*	

n - number of determinations

The values are expressed as mean \pm coefficient of variation, μ g free steroid in 2 ml aliquots from a pooled plasma sample.

*The stationary phase, NCS, does not separate DHA and EPI.

**Aetiocholanolone could not be detected in 2 ml of plasma pool A.

Precision.

The precision of the method was determined by performing replicate analyses on two plasma pools. In Table 23, the results obtained in the analyses of these pools are given. From the values given in this table, it can be seen that the precision of the method is similar for all of the four steroids determined, covering a wide concentration range. The mean coefficient of variation was $6\% \pm 2.8$ (S.D.) with a range extending from 2 - 12%.

Accuracy.

The accuracy of the described method for the quantitative determination of the solvolizable conjugates of androsterone, aetiocholanolone, DHA and epiandrosterone in human plasma was tested by adding synthetic sulphate conjugates of these steroids into samples from plasma pools and determining the amount of each steroid recovered after the whole procedure. The endogenous amount of each of these steroids was first determined several times in the pools used for recovery experiments. In Table 24, the recoveries of added steroid sulphate conjugates are given. The average recoveries were: androsterone $96\% \pm 8$ (mean \pm S.D.), DHA $94\% \pm 7$, epiandrosterone $94\% \pm 7$.

In the current study, aetiocholanolone could not be detected in the fraction of solvolizable steroids in this pool. The average recovery of synthetic aetiocholanolone sulphate added to plasma from pool A was $94\% \pm 7$.

Table 23

REPLICATE ANALYSES OF PLASMA POOLS

Pool	n	A	<u>Steroids</u>		
			E	DHA	EPI
A	7	13.0 \pm 11%	-	37.0 \pm 8%	5.5 \pm 3%
A	5	10.0 \pm 10%	-	35.0 \pm 5%	5.2 \pm 3%
B	6	72.0 \pm 6%	31.0 \pm 5%	101.0 \pm 8%	83.0 \pm 6%
B	8	73.1 \pm 2%	28.0 \pm 12%	100.4 \pm 3%	71.2 \pm 6%
B	6	74.0 \pm 6%	30.0 \pm 9%	105.0 \pm 4%	75.6 \pm 6%

n - number of determinations

Replicate analyses of two plasma pools. Pregnenolone sulphate was added as internal standard and the steroids were taken through the procedure, as described. The final extracts were chromatographed on 3% QF-1. The results are expressed as μg of free steroid per 100 ml of plasma pool \pm % S.D.

Table 24

RECOVERIES OF STEROID SULPHATE CONJUGATES ADDED TO PLASMA

Steroid	Initial concn. μg free steroid/ 2 ml aliquot	μg Steroid sulphate added	Equivalent μg free steroid added	μg Free steroid found	% Recovery
ANDROSTERONE	0.26	1	0.68	1.00	109
		2	1.35	1.46	89
		5	3.39	3.39	89
		10	6.78	7.00	99
		15	10.18	9.79	94
		Mean Recovery			96% \pm 8
AETIOCHOLANOLONE	0	1	0.68	0.62	91
		2	1.35	1.25	93
		5	3.39	3.02	89
		10	6.78	6.61	97
		15	10.18	10.00	98
		Mean Recovery			94% \pm 4
DEHYDROEPIANDROSTERONE	0.74	1	0.68	1.46	106
		2	1.35	1.96	91
		5	3.38	3.94	95
		10	6.76	6.85	90
		15	10.10	9.85	90
		Mean Recovery			94% \pm 7
EPIANDROSTERONE	0.11	1	0.68	0.81	103
		2	1.35	1.46	100
		5	3.38	3.14	90
		10	6.76	6.08	88
		15	10.18	9.38	91
		Mean Recovery			94% \pm 7

The steroid conjugates in ethanol were added to 2 ml aliquots of plasma from Pool A. The values are expressed as μg of the free steroid per 2 ml aliquot.

The recovery from each step in the procedure of DHAS sodium salt was determined using tritiated DHAS. The recoveries of radioactivity in the individual steps of the procedure were as follows: (mean \pm S.D.): Sephadex column chromatography 79% \pm 6; extraction + Sephadex column chromatography and solvolysis 74% \pm 3; the total procedure 65% \pm 3 (Table 25a).

Table 25b shows the results for the recovery of radioactivity when tritiated pregnenolone sulphate was added to plasma and taken through the whole procedure. These absolute recoveries of 62% \pm 5 and 67% \pm 3 for pregnenolone sulphate were satisfactory and agreed with that obtained for DHAS.

Sensitivity.

In the present method, 2 ml of plasma were used in each analysis. The usual amount injected into the gas chromatograph and the usual setting of the attenuator allowed the measurement by planimetry of epiandrosterone TMSE peak on the QF-1 column when the concentration of this steroid was greater than 5 μ g/100 ml plasma. This peak was the last one of the quantitated steroids to emerge from the column, thus having a least favourable form for measurement of peak height. The sensitivity of the method could be increased by initially using a larger volume of plasma, or by increasing the amount of the final solution of the steroid TMSE derivatives which is used for g.l.c. In the majority of analyses, only 20% of the final solution was used for g.l.c.

Table 25a

RADIOACTIVE RECOVERIES AFTER ADDITION OF 34×10^3 d.p.m.

DHA- 7α - ^3H SULPHATE SODIUM SALT TO HUMAN PLASMA

PROCEDURE Recovery after	n	% RECOVERY Mean \pm S.D.
Sephadex Column Chromatography	6	92 \pm 4
Extraction + Sephadex Col. Chromatography	6	79 \pm 6
Extraction + Sephadex Col. Chromatography + Solvolysis	4	74 \pm 3
Total Procedure	5	65 \pm 3

Table 25b

RADIOACTIVE RECOVERIES AFTER ADDITION OF 44×10^3 d.p.m.

PREGNENOLONE- 7α - ^3H SULPHATE AMMONIUM SALT TO HUMAN PLASMA

RECOVERY AFTER	n	% RECOVERY Mean \pm S.D.
Total Procedure	6	62 \pm 5
" "	6	67 \pm 3

n - number of determinations

As stated earlier, the gas-liquid chromatograph has the potential for much greater sensitivity, but the use of very high sensitivities puts great demands on the purity of the solvents and reagents used in the method. Using the solvents recorded in the materials section of this procedure and 5 ml of plasma, the lower limit of detection of epiandrosterone sulphate in plasma was 2 $\mu\text{g}/100\text{ ml}$.

SECTION VII

PLASMA LEVELS OF 17-OXOSTEROID SULPHATES

IN NORMAL MALES AND FEMALES

A. INVESTIGATION OF THE LEVELS OF 17-OXOSTEROID SULPHATES
IN THE PERIPHERAL PLASMA OF NORMAL MEN AND WOMEN.

As with the control group for the urinary studies, this group of normal subjects was made up of members of staff, friends and ambulant patients who were free from any disorders which might have an effect on the levels of the 17-OS sulphates in plasma. These disorders have been mentioned previously in Section V.

Blood samples (10 ml) were obtained from fifty-three normal males whose ages ranged from 4 years to 72 years, and thirty-nine normal females whose ages ranged from 16 years to 51 years. These blood samples were drawn off between 9.00 a.m. and 11.00 a.m., transferred into heparinized tubes and centrifuged immediately. The plasma was stored at -20° until analysed. The effect of storage at -20° for different lengths of time on the concentrations of the individual steroids was investigated. This was undertaken by analysing six fresh samples from a newly formed fresh plasma pool, transferring the remaining plasma in 2 ml aliquots into small plastic containers and storing at -20° for different periods of time up to four months. After storage at -20° , these 2 ml plasma samples were thawed and analysed. The results are presented in Table 26. Using Student's 't'-test, no statistical significant difference was found on comparing the concentrations of the steroids in the fresh samples with those that had been stored at -20° . Neither was the length of time of

Table 26

EFFECT OF STORAGE AT -20° ON THE CONCENTRATIONS OF THE 17-O5 SULPHATES

IN A PLASMA POOL

Period of Storage	n	A Mean \pm S.D.	E Mean \pm S.D.	DHA Mean \pm S.D.	EPI Mean \pm S.D.
Fresh	6	1.40 \pm 0.13	1.00 \pm 0.07	1.47 \pm 0.08	0.39 \pm 0.03
7 days	4	1.43 \pm 0.05	0.94 \pm 0.03	1.45 \pm 0.05	0.35 \pm 0.02
Difference		not significant	not significant	not significant	not significant
1 month	6	1.37 \pm 0.08	0.95 \pm 0.06	1.42 \pm 0.06	0.39 \pm 0.04
Difference		not significant	not significant	not significant	not significant
3 months	4	1.38 \pm 0.03	0.98 \pm 0.06	1.39 \pm 0.11	0.38 \pm 0.02
Difference		not significant	not significant	not significant	not significant

n - number of samples analysed.

Values expressed as μg free steroid per 2 ml aliquot.

Student's 't' test was used to compare the concentrations of the steroids in the plasma after different periods of storage at -20° with the concentrations of these steroids in fresh plasma.

storage found to have an effect on the concentrations of these 17-OS sulphates.

Each plasma sample from the ninety-two normal subjects was analysed once, and approximately one-third of the samples were analysed in duplicate. The results are presented in Tables 27a - 27e for females, and in 28a - 28h for males. Figure 31 shows a typical g.l.c. tracing of the steroid sulphates from the plasma of a normal male. To study the possible effect of age on the concentration of the 17-OS sulphates, the normal subjects were divided into the following age groups:

- 1) age 0 - 9 years - 3 males, 0 females
- 2) age 10 - 19 years - 2 males, 6 females
- 3) age 20 - 29 years - 19 males, 12 females
- 4) age 30 - 39 years - 7 males, 9 females
- 5) age 40 - 49 years - 12 males, 8 females
- 6) age 50 - 59 years - 6 males, 4 females
- 7) age 60 - 69 years - 3 males, 0 females
- 8) age 70 - 79 years - 1 male, 0 females.

The mean levels and standard deviations of each individual steroid were calculated for each group. The maximum mean levels of all four steroids measured mostly occurred in the 20 - 29 years age-group in both sexes, and thereafter they all showed a significant decline with increasing age. These findings agree with those reported by Migeon et al. (1957), De Neve and Vermeulen (1965), Feher et al. (1966) and Wang et al. (1968). The concentrations of the four 17-OS sulphates

Table 27a

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL FEMALES

(μ g, Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
1	16	12.2	2.1	51.9	9.2	75.4
2	16	43.3	0	89.8	14.0	147.1
3	17	23.1	3.6	69.2	7.6	103.5
4	18	18.9	5.2	46.8	9.3	80.2
5	19	55.6	1.9	66.2	11.2	134.9
6	19	21.8	4.6	51.0	9.6	87.0
Range	16-19	12.2-55.6	0-5.2	46.8-89.8	7.6-14.0	75.4-147.1
Mean	17.5	29.1	2.9	62.4	10.1	104.6
S.D.	1.3	16.6	1.9	33.6	2.2	47.6

Table 27b

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL FEMALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
7	20	121.4	4.2	160.4	30.8	316.8
8	20	79.3	7.5	109.6	23.6	220.0
9	20	194.2	10.0	211.5	46.4	462.0
10	20	129.0	11.9	145.8	39.2	325.6
11	22	40.6	13.4	94.3	9.7	158.0
12	22	28.4	10.2	78.1	11.1	127.8
13	25	34.9	10.8	91.0	9.7	146.4
14	26	45.9	5.9	140.7	16.1	208.5
15	26	23.3	3.7	74.2	16.5	117.7
16	26	68.5	6.3	165.7	22.4	262.9
17	28	10.5	0	62.2	9.6	82.3
18	29	40.2	5.6	89.6	13.6	148.0
Range	20-29	10.5-194.2	0-13.4	62.2-211.5	9.6-46.4	82.3-462.0
Mean	23.6	68.0	7.4	118.5	20.9	214.6
S.D.	3.3	54.4	3.9	45.6	12.1	110.0

Table 27c

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL FEMALES

(μg Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
19	30	43.6	5.9	46.4	14.5	110.4
20	30	39.3	1.5	94.0	20.3	155.1
21	31	4.5	0	41.9	8.8	55.2
22	33	25.6	2.0	42.3	13.6	83.5
23	35	36.6	3.8	49.2	15.0	104.6
24	36	22.3	3.4	58.9	5.2	89.8
25	38	44.2	3.6	98.6	13.9	160.3
26	39	16.6	4.2	46.2	11.3	78.3
27	39	50.6	6.8	89.9	13.9	161.2
Range	30-39	4.5-50.6	0-6.8	41.9-98.6	5.2-20.3	55.2-161.2
Mean	34.0	31.4	3.4	63.0	12.9	110.9
S.D.	3.5	15.1	2.1	25.2	4.2	39.2

Table 27d

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL FEMALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
28	40	16.5	7.0	18.1	10.5	52.1
29	41	32.4	0	59.8	2.6	94.8
30	42	41.9	1.8	50.6	3.0	97.3
31	44	16.1	4.2	46.4	10.3	77.0
32	46	11.1	0	26.1	0	37.2
33	47	19.8	0	30.2	2.0	52.0
34	48	22.6	0	84.9	19.2	126.7
35	49	5.0	2.6	16.8	10.8	35.2
Range	40-49	5.0-41.9	0-7.0	16.8-84.9	0-19.2	35.2-126.7
Mean	44.6	20.6	1.9	41.6	7.3	71.5
S.D.	3.3	11.7	2.5	23.4	6.4	32.8

Table 27e

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL FEMALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
36	50	35.7	4.8	69.3	10.3	120.1
37	50	18.8	8.2	21.7	11.0	59.7
38	51	5.1	0	8.6	2.0	15.7
39	51	8.0	0	12.9	4.6	25.5
Range	50-51	5.1-35.7	0-8.2	8.6-69.3	2.0-11.0	15.7-120.1
Mean	50.5	16.7	3.2	28.1	6.9	55.2
S.D.		13.8	4.0	27.9	4.3	47.1

Table 28a

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
1	4	3.6	0	0	9.1	12.7
2	7	11.6	0	8.0	5.4	25.0
3	10	35.8	5.7	59.8	21.2	122.5
Range	4-10	3.6-35.8	0-5.7	0-59.8	5.4-21.2	12.7-122.5
Mean	7	17.0	1.9	22.6	11.9	53.4
S.D.	3	16.7	3.3	32.5	8.3	60.2

Table 28b

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
4	13	53.5	0	44.9	20.2	118.0
5	14	65.4	7.7	95.8	23.2	192.1
Range	13-14	53.5-65.4	0-7.7	44.9-95.8	20.2-23.2	118.0-192.1
Mean	13.5	59.5	3.9	70.4	21.7	155.0
S.D.	0.7	8.4	5.4	36.0	2.1	52.4

Table 28c

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(µg Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
6	22	101.9	5.7	276.8	21.2	405.6
7	22	108.4	5.6	322.0	33.5	469.5
8	22	98.0	3.3	181.1	33.2	315.6
9	23	28.0	0	58.8	11.2	98.0
10	23	38.9	0	112.4	7.0	158.3
11	23	69.1	9.5	355.5	17.8	451.9
12	24	31.6	0	72.1	15.4	119.1
13	24	96.5	21.3	144.7	15.2	277.7
14	24	72.6	6.7	217.3	15.5	312.1
15	25	108.7	13.3	204.6	38.9	365.5
16	25	32.1	0	110.3	6.8	149.2
17	25	76.0	17.0	158.2	14.1	265.9
18	26	34.3	0	176.6	16.8	227.7
19	27	65.4	2.9	154.8	24.6	247.7
20	27	92.8	9.0	168.2	14.7	284.7
21	28	37.9	0	70.2	11.2	119.3
22	28	61.1	9.6	176.2	7.3	254.2
23	28	62.3	9.4	164.3	11.9	247.9
24	28	113.5	7.8	350.5	30.0	501.8
Range	20-29	28.0-113.5	0-21.3	58.8-355.5	6.8-38.9	98.0-501.8
Mean	24.9	69.9	6.4	182.9	18.2	277.5
S.D.	2.2	29.8	6.2	88.8	9.6	120.7

Table 28d

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
25	32	39.9	3.4	97.1	13.9	154.3
26	35	50.7	6.2	108.9	8.9	174.7
27	35	49.6	15.0	107.3	8.7	181.6
28	34	87.7	9.3	141.7	13.9	244.6
29	34	81.1	9.2	152.1	15.9	259.3
30	37	18.1	6.0	18.1	12.3	54.4
31	37	55.5	7.0	110.4	23.9	196.8
Range	30-39	18.1-87.7	3.4-15.0	18.1-152.1	8.7-23.9	54.5-259.3
Mean	34.8	53.5	8.0	105.1	13.9	180.6
S.D.	1.7	22.0	3.7	43.3	5.1	67.2

Table 28e

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μg Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
32	42	50.6	10.2	80.3	5.8	146.8
33	43	39.5	4.3	33.6	14.0	94.4
34	44	39.7	0	98.1	11.9	149.4
35	45	20.6	5.7	44.9	14.0	85.2
36	45	51.9	0	86.2	15.2	163.3
37	47	30.7	9.5	99.0	16.5	155.7
38	47	18.5	0	26.8	15.8	62.1
39	48	58.2	0	107.1	13.6	178.9
40	48	31.0	0	95.7	14.2	140.9
41	49	20.6	0	38.9	14.9	74.4
42	49	39.4	0	45.6	15.2	100.2
43	49	41.6	0	107.3	18.2	167.1
Range	40-49	18.5-58.2	0-10.2	26.8-107.3	5.8-18.2	62.1-178.9
Mean	46.5	36.9	2.5	72.0	14.4	126.5
S.D.	2.7	12.9	3.9	31.2	3.2	40.5

Table 28f

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μ g Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
44	51	22.3	6.0	17.3	12.3	57.9
45	51	76.9	4.4	132.0	25.0	238.3
46	52	20.2	0	52.9	2.8	76.0
47	52	29.3	0	58.7	2.8	91.0
48	52	32.1	0	56.6	5.6	94.0
49	59	20.6	5.9	24.4	15.3	66.3
Range	50-59	20.2-76.9	0-6.0	17.3-132.0	2.8-25.0	57.9-238.3
Mean	52.8	33.6	2.7	57.0	10.6	103.9
S.D.	2.8	21.8	3.0	40.8	8.7	67.3

Table 28g

LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μg Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
50	61	31.8	3.7	75.3	11.3	122.1
51	62	17.3	0	58.3	13.2	88.8
52	69	5.6	0	0	8.4	14.0
Range	60-69	5.6-31.8	0-3.7	0-75.3	8.4-13.2	14-122.1
Mean	64.0	18.2	1.2	44.5	12.0	74.8
S.D.	4.3	13.1	2.1	39.5	10.5	55.4

Table 28h

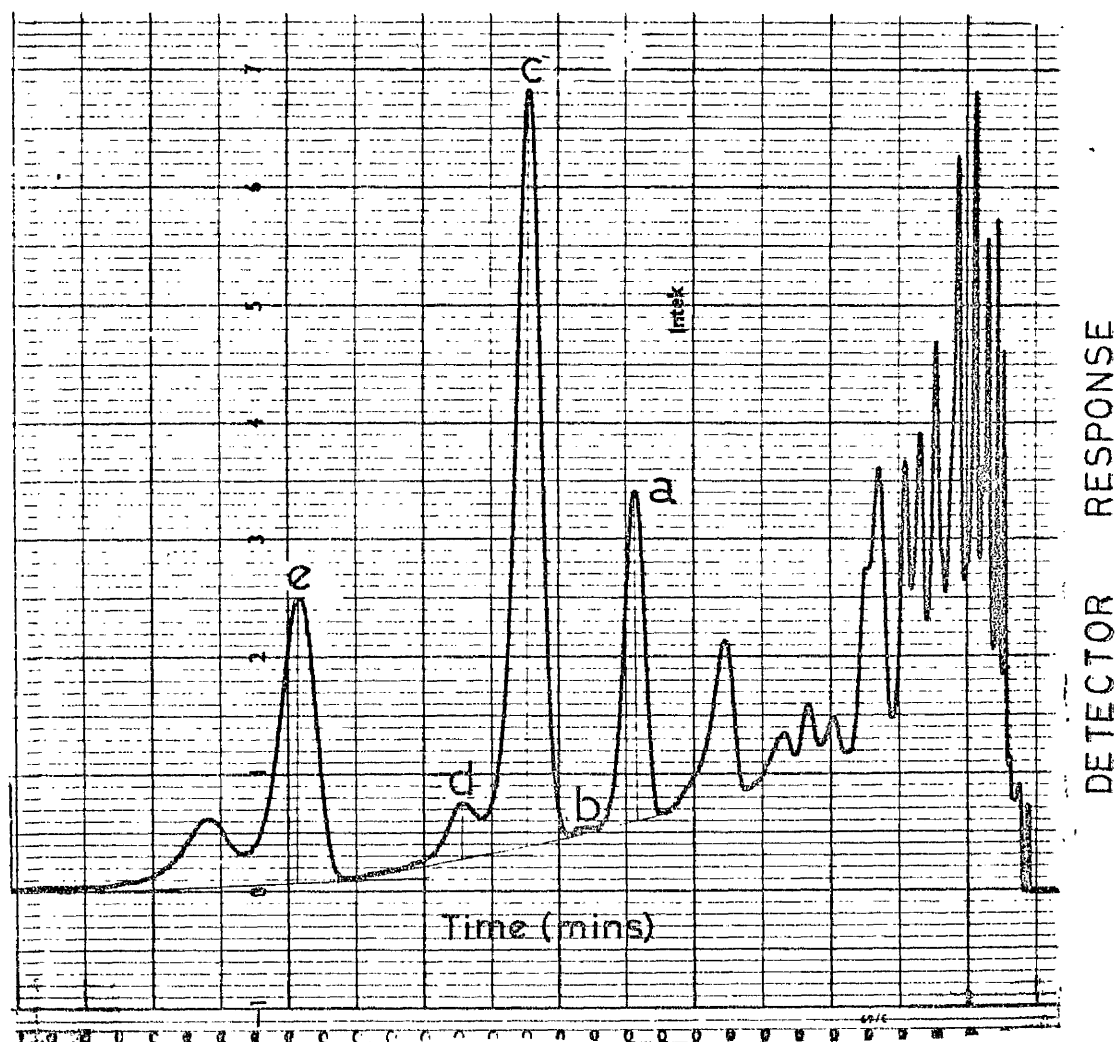
LEVELS OF PLASMA 17-OXOSTEROID SULPHATES IN NORMAL MALES

(μg Free Steroid/100 ml Plasma)

Subject	Age	A	E	DHA	EPI	TOTAL
Overall Range	4-72	3.6-113.5	0-21.3	0-355.5	2.8-38.9	14.0-501.8
Overall Mean	36	48.4	4.5	109.1	15.2	177.9
S.D.	15.6	29.5	5.1	83.2	7.5	115.5

GLC tracing of the 17-oxosteroid sulphates
from normal male plasma

Column 3% QF1



Typical male plasma levels of 17-OS sulphates with
DHA (c) > androsterone (a) > epiandrosterone(d) > aetio(b)

100

were shown to vary widely among different normal subjects. The plasma concentrations of the individual 17-OS sulphates, which have been reported by other authors, have also shown considerable variation, and generally these values for the concentrations of the sulphate conjugates of androsterone, aetiocholanolone, DHA and epiandrosterone are in agreement with those previously reported (Table 29).

Table 29

COMPARISON OF REPORTED CONCENTRATIONS OF 17-OS SULPHATES
IN PERIPHERAL HUMAN PLASMA

Author(s)	n	A	E	DHA	EPI	TOTAL
Present Report	53M	4-108	0-21	0-355	2-33	12-502
	39F	4-194	0-13	8-212	0-20	15-462
Wang <u>et al.</u> (1968)	41M	4-104	-	12-392	-	-
	51F	5-129	-	10-248	-	-
Vihko (1966)	62M	2-107	-	19-323	0-32	-
	54F	0-72	-	0-188	0-17	-
Baulieu (1965a)	6M	0-23	0-23	40-150	-	-
	4F	0-85	0-8	36-130	-	-
De Neve and Vermeulen (1965)	9M	17-53	3-25	40-73	-	53-260
	3F	26-30	5-24	49-65	-	72-88
Oertel and Kaiser (1962)	6M	24-64	7-41	67-158	-	-
	6F	23-46	16-34	44-106	-	-

The values are expressed as μg of the free steroid/100 ml plasma.

n - number of subjects studied.

M - indicates male subject.

F - indicates female subject.

B. EFFECT OF AGE AND SEX ON THE CONCENTRATION OF THE
17-OXOSTEROID SULPHATES IN PLASMA.

The concentrations of all the steroid sulphates analysed significantly decreased with advancing age in both sexes. It must be noted, however, that there were large variations, and in the youngest and oldest age groups of both sexes there were subjects having roughly similar plasma steroid concentrations. The statistical significance of this decline of concentration with age was variable from steroid to steroid and between the sexes (Table 30). The most significant decline was shown by AS ($p < 0.001$) and DHAS ($p < 0.005$) in males, and by EPIS ($p < 0.005$) in females. The changes in the plasma levels of these 17-OS sulphates are in accord with the results reported earlier in this thesis for the urinary excretion pattern of these steroids.

Sex differences in the peripheral plasma concentrations of some of these 17-OS sulphates have been reported by De Neve and Vermeulen (1965) (total 17-OS), Feher et al. (1966) (DHA and A) and Wang et al. (1968) (DHA), the values for males being higher. In this present study, the differences in the concentrations of the steroids between the sexes were statistically significant only for DHA ($p < 0.01$) and for the total plasma 17-OS sulphate fraction ($p < 0.025$), when the entire groups of males and females were compared. When the concentrations of the steroids from the individual age groups were compared between the sexes, the differences were variable.

Table 30

STATISTICAL SIGNIFICANCE OF THE DECLINE WITH INCREASING
AGE IN THE CONCENTRATIONS OF EACH OF THE STEROIDS

Steroids	A	E	DHA	EPI	TOTAL
Male	$p < 0.001$	$p < 0.02$	$p < 0.005$	$p < 0.05$	$p < 0.005$
Female	$p < 0.025$	$p < 0.02$	$p < 0.02$	$p < 0.005$	$p < 0.02$

In Table 31, the significance criteria for the differences between the two sexes in different age groups are given. In each case where there was a significant difference between the sexes, it was due to the higher mean concentration of the steroid in the male group. From this table, it can be observed that the differences of the steroid concentrations between the sexes were more marked in the middle age groups rather than in the younger or older groups. For instance, the levels of DHAS for males were significantly greater than those for females throughout the 20 - 49 years age range, but in the youngest and oldest group there was no significant difference. However, it must again be noted that the variations in the concentrations are very large in both sexes, and a great deal of overlap exists.

Table 31

STATISTICAL SIGNIFICANCE OF SEX DIFFERENCES IN THE CONCENTRATIONS OF EACH
OF THE STEROIDS IN THE ENTIRE GROUP, AND IN THE AGE GROUPS

Age Group	A	E	DHA	EPI	TOTAL
Entire Group	Not significant	Not significant	$p < 0.01$	Not significant	$p < 0.025$
10 - 19	$p < 0.025$	Not significant	Not significant	$p < 0.001$	Not significant
20 - 29	Not significant	Not significant	$p < 0.02$	Not significant	Not significant
30 - 39	$p < 0.05$	$p < 0.02$	$p < 0.05$	Not significant	$p < 0.05$
40 - 49	$p < 0.01$	Not significant	$p < 0.025$	$p < 0.02$	$p < 0.005$
50 - 59	Not significant	Not significant	Not significant	Not significant	Not significant

C. INVESTIGATION OF DIURNAL VARIATION IN THE PLASMA
CONCENTRATIONS OF THE 17-OXOSTEROID SULPHATES.

The concentrations of the 17-OS sulphates were examined in morning and evening plasma samples from twelve of the normal male control subjects. The first specimen of blood was drawn off at 9.00 a.m. and the evening specimen at 5.00 p.m. The variations in the plasma levels are shown in Table 32. Due to the large individual variations of the concentrations of the steroids in these control subjects, it was not possible to apply a statistical test to the average levels obtained in the morning and evening samples. For this reason the various steroid values obtained in one subject were compared to his 9.00 a.m. values, and the percentage increase or decrease of the 5.00 p.m. concentrations of the steroids were calculated. In Table 32, increases in the steroid level in the evening sample are indicated by a + sign, and decreases by a - sign. Examination of this Table reveals no conclusive pattern in the plasma level of any individual steroid. Eight out of the twelve subjects demonstrated a decrease in the concentration of AS, ranging from 2.2% to 31%, with a mean of $12.5\% \pm 6.7$ (S.D.). In the remaining four normal subjects, the concentration of AS rose with a mean value of $6.7\% \pm 5.3$ (S.D.). The concentration of DHAS fell in nine out of the twelve subjects by a mean value of $6.7\% \pm 5.9$ (S.D.). The mean rise registered in the other three subjects was $17.1\% \pm 8.6$ (S.D.). These results are not in agreement with those reported by other workers.

COMPARISON OF THE CONCENTRATIONS OF THE 17-OXOSTEROID SULPHATES
IN MORNING AND EVENING PLASMA SAMPLES

Subject	Age	A		E		DHA		EPI	
		9 a.m.	5 p.m.	9 a.m.	5 p.m.	9 a.m.	5 p.m.	9 a.m.	5 p.m.
1	22	104.5	96.8	2.6	0	282.4	280.6	23.6	22.0
Difference		-7.4%		-100%		-0.6%		-6.8%	
2	23	39.1	37.6	0	0	114.0	110.7	7.0	7.0
Difference		-3.8%		-		-2.9%		0%	
3	24	96.5	107.6	21.3	26.3	144.7	118.3	15.2	11.0
Difference		+11.5%		+23.5%		-18.2%		-22.4%	
4	27	92.8	66.1	9.0	8.5	168.2	162.2	14.7	16.4
Difference		-28.8%		-5.6%		-3.6%		+11.6%	
5	28	61.1	61.4	9.6	10.6	176.2	212.2	7.3	9.2
Difference		+0.5%		+10.4%		+20.4%		+26.0%	
6	28	37.9	42.0	0	3.5	70.2	86.8	11.2	12.7
Difference		+10.8%		+100%		+23.6%		+13.4%	
7	28	118.7	101.9	12.7	5.7	276.8	249.0	21.2	15.5
Difference		-14.1%		-55.1%		-10.0%		-26.9%	
8	28	113.5	110.0	7.8	24.5	350.5	311.8	30.0	23.5
Difference		-3.1%		+214%		-11.0%		-21.7%	
9	34	78.7	81.8	9.3	9.2	141.7	152.1	13.9	15.9
Difference		+4%		-10.7%		+7.3%		+14.4%	
10	35	50.7	49.6	6.2	15.0	108.9	107.3	8.9	8.7
Difference		-2.2%		+141.9%		-1.5%		-22.5%	
11	42	56.1	50.6	11.0	10.2	82.6	80.2	5.9	5.8
Difference		-9.8%		-7.3%		-2.9%		-16.9%	
12	52	29.3	20.2	0	0	58.7	52.9	2.8	2.8
Difference		-21.0%		-		-9.9%		-0%	

Results expressed as μg free steroid/100 ml plasma. The differences are expressed as a percentage of the concentration at 9.00 a.m.

Laatikainen and Vihko (1968) reported that the plasma concentrations of monosulphated DHA and androsterone, mono- and disulphated androst-5-ene-3 β ,17 β -diol and pregn-5-ene-3,20 α -diol have a diurnal variation which is similar for all of these conjugates, and follows a pattern in which the lowest plasma concentrations were observed at midnight or at 8.00 a.m. and the highest at noon or at 4.00 p.m. In contrast to this, Migeon et al. (1957) found that the highest concentrations of DHAS and AS were observed in the morning, with a decrease during the day. A similar pattern has been observed for the plasma concentration of cortisol by various researchers, including Migeon et al. (1962), Martin et al. (1963) and Silverberg et al. (1968). Using a frequent sampling technique, Weitzman et al. (1971) reported that the period during the three hours before awaking and the first hour of wakefulness represented the most intense time of adrenal-cortical activity and accounted for nearly half of the total cortisol secreted over 24 hours. In the following investigations of the concentrations of these 17-OS sulphates in the plasma of normal and porphyric patients, the blood samples were drawn off in the morning whenever possible.

D. INVESTIGATION OF THE EFFECT OF THE MENSTRUAL CYCLE ON
THE PLASMA CONCENTRATIONS OF THE 17-OXOSTEROID SULPHATES.

The effect of the menstrual cycle on the levels of the sulphates of androsterone, aetiocholanolone, DHA and epiandrosterone in the plasma of four normal women with regular menstruation was investigated. Blood samples were obtained at 9.00 a.m. on various days throughout the complete menstrual cycle. The heparinized blood was centrifuged immediately and the plasma stored at -20° until the specimens were analysed in random order. The results are presented in Table 33. As repeatedly demonstrated, there were great differences in the peripheral plasma concentrations of these compounds between individual subjects. However, in the same subject the concentrations of the individual steroids were very similar in the samples collected throughout the cycle and, using Student's 't'-test, no statistically significant difference in the levels of the steroids in the different phases of the menstrual cycle could be observed. Therefore, it was concluded that the concentrations of the plasma 17-OS sulphates are not dependent on the phase of the menstrual cycle. The biphasic pattern in the peripheral plasma concentrations of DHAS reported by Kumari et al. (1969) was not observed. The results obtained in this investigation were confirmed in 1971 by Laatikainen and Vihko, who reported that the concentrations of AS, DHAS and EPIS determined on the 10th and 22nd days of the menstrual cycle did not differ significantly.

These assiduous investigations into the levels of the neutral 17-oxosteroid conjugates in the urine and the plasma

Table 33

EFFECT OF THE MENSTRUAL CYCLE ON THE LEVELS OF PLASMA 17-OS SULPHATES IN FOUR NORMAL FEMALES

Subject	Age	A		E		DHA		EPI	
		1*	2*	1	2	1	2	1	2
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
1	20	193.6 \pm 26.5 (3)	194.6 \pm 13.7 (5)	10.5 \pm 1.8	9.6 \pm 1.7	208.9 \pm 33.1	215.0 \pm 17.7	46.7 \pm 7.4	46.2 \pm 2.7
Difference		Not significant		Not significant		Not significant		Not significant	
2	20	80.4 \pm 5.0 (4)	78.3 \pm 14.3 (5)	7.8 \pm 0.8	7.3 \pm 1.3	99.4 \pm 3.4	118.1 \pm 8.0	21.6 \pm 1.8	25.6 \pm 4.3
Difference		Not significant		Not significant		p < 0.01		Not significant	
3*	26	46.0 \pm 3.3 (5)	45.8 \pm 4.4 (4)	4.6 \pm 2.6	7.0 \pm 2.4	134.3 \pm 9.7	147.0 \pm 16.7	16.8 \pm 0.8	15.4 \pm 1.4
Difference		Not significant		Not significant		Not significant		Not significant	
4	20	123.8 \pm 2.0 (5)	135.4 \pm 14.8 (4)	9.9 \pm 2.1	14.4 \pm 2.2	138.8 \pm 16.7	154.4 \pm 9.8	37.5 \pm 5.2	41.2 \pm 4.9
Difference		Not significant		Not significant		Not significant		Not significant	

Values in parenthesis indicate the number of samples (one per day) analysed during that period of the menstrual cycle.

1* = First portion of the menstrual cycle, including the menstrual and the follicular phase.

2* = Second portion of the menstrual cycle, including the ovulatory and the luteal phase.

of a large number of normal individuals, the effects of age, sex, time of day and the phase of the menstrual cycle on these levels, established a firm background of knowledge of the normal levels and excretory patterns of these 17-OS conjugates. This provided a strong spring-board for researching into the abnormal deviations in steroid excretion which have been reported in patients suffering from acute intermittent porphyria and the extension of these investigations to include patients suffering from other types of porphyria.

SECTION VIII

STEROIDAL STUDIES IN PATIENTS

WITH HEPATIC PORPHYRIA

A. INTRODUCTION.

The subjects of this study consisted of fifty-five patients suffering from several different types of hepatic porphyria, who were obtained from various parts of the United Kingdom. There were thirty-four patients with acute intermittent porphyria, fourteen patients with porphyria cutanea tarda (symptomatica), five patients with hereditary coproporphyria and two patients with variegate porphyria. The diagnosis of the type of porphyria in every case was based on the criteria and classification given by Goldberg (1971). The urinary 17-OS sulphate and glucuronide levels and the 17-OS sulphate levels in the plasma were estimated in these male and female porphyric patients and compared with the corresponding levels obtained for normal males and females. For many of these patients, it was only possible to obtain one complete 24-hour urine specimen, and thus statistical data is not available for every patient. In the event of steroid estimations being made on a single urine specimen, elevations were noted if the level of the steroid was greater than that found for the upper limit of the normals of that particular age group. It was also not possible to obtain a plasma sample from every patient whose urine was investigated. In addition to these steroid studies, the urine porphyrins and precursors of these porphyric patients were estimated.

Measurement of urinary δ -aminolaevulinic acid, porphobilinogen and porphyrins.

Urinary δ -aminolaevulinic acid (ALA) and porphobilinogen (PBG)

were measured in 24-hour collections of urine by the method of Mauzerall and Granick (1956). Urinary uroporphyrin and coproporphyrin were measured on samples of the same collections of urine by the method of Rimington (1961). These analyses were carried out in the Department of Materia Medica, Stobhill General Hospital, Glasgow, by G.G. Thompson, by whose permission I produce these results. Normal human levels of urinary porphyrins, ALA and PBG have been recorded by Goldberg (1966) and Moore et al. (1972a).

B. STEROIDAL STUDIES IN FEMALES WITH ACUTE INTERMITTENT
PORPHYRIA.

The total number of female acute intermittent porphyric patients studied was twenty-three, of whom only four were in attack, eighteen were in remission for periods ranging from two years to twenty years and one was a latent AIP. Estimation of the steroids in the plasma and urines of these porphyric patients revealed fifteen patients who had abnormally high concentrations of several of the 17-OS conjugates either in the plasma or the urine (Tables 34a and 34b). In most of these subjects, these abnormally high concentrations of steroids were observed in both the urine and the plasma. Unfortunately, in some of the cases it was not possible to procure both a plasma specimen and a 24-hour urine specimen.

The most striking steroid abnormalities detected in the majority of the patients were the increased excretions of DHA and aetiocholanolone sulphates and aetiocholanolone glucuronide in the urine, and the elevated levels of DHAS in the plasma. Figures 32 and 33 depict typical g.l.c. traces of urinary and plasma steroids from female patients with AIP. In the g.l.c. trace of the urinary sulphate fraction in figure 32, the large peak (c) due to excessive amounts of DHA can be observed. Similarly, in figure 33, the large peak (b) is due to excessive quantities of aetiocholanolone sulphate in the plasma. Increases in the excretions of androsterone and epiandrosterone sulphates, and androsterone and 11-oxy-17-OS glucuronides were

17-OXOSTEROID AND PORPHYRIN LEVELS IN FEMALE PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA

Subject	Age	Attack or Remission	n	Urinary Sulphates (mg/24 hr.)					Urinary Glucuronides (mg/24 hr.)					Total Urinary	Plasma Sulphates (µg/100 ml.)					Total Plasma	Urine					
				E/A	A	E	DHA	EPI	A	E	DHA + EPI	11-OA	11-OE		11-OHA	11-OHE	17-OS	A	E		DHA	EPI	17-OS	ALA	PBG	Copro
Normal Controls	Range 20-29	Normal	15	0.47-1.35	0-0.89	0.03-0.55	0-0.78	0-0.32	1.51-4.43	1.46-4.00	0-0.47	0.07-0.27	0.15-1.54	0.29-1.38	0-1.00	5.5-10.9	10-195	0-12	62-211	9-47	82-317					
Case 1	24	R	1	2.31	0.59	1.56	2.00	1.77	1.10	2.35	0.30	0.19	0.74	0.60	0.32	11.5	299	436	608	49	1392	A	A	A	A	A
Case 2	24	R	1	1.78	0.57	0.89	1.05	0.92	0.35	0.75	0.08	0.09	0.36	0.21	0.14	5.4	296	192	557	40	1085	A	A	A	A	A
Case 3	23	A	6*	2.20	0.49	0.92	2.51	0.39	0.30	0.84	0.18	0.05	0.41	0.13	0.31	6.5	234	295	243	54	827	A	A	A	A	A
Case 4	25	A	1	2.31	0.48	1.22	2.62	0.56	1.87	4.22	0.56	0.22	1.16	0.92	0.47	14.3	86	10	398	39	533	A	A	A	A	A
Case 5	28	A	4*	1.52	0.43	0.33	2.69	0.96	3.22	5.22	1.40	0.25	1.57	1.34	1.44	18.9	-	-	-	-	-	A	A			
Normal Controls	Range 30-39	Normal	11	0.63-2.16	0.02-0.96	0.06-0.38	0.01-0.80	0.04-0.33	0.16-1.96	0.30-2.41	0-0.32	0-0.27	0.20-1.04	0.24-1.38	0-0.75	1.6-7.5	4-51	0-7	41-99	5-21	55-160					
Case 7	31	R	1	2.83	0.26	0.68	0.55	0.77	1.37	3.94	0.39	0.33	1.09	0.66	0.52	10.6	-	-	-	-	-	A	A	A	A	A
Case 8	32	A	1	2.70	1.05	0.60	4.03	0.88	0.45	3.46	0.24	0.08	0.39	0.30	0.34	11.8	145	22	229	22	418	A	A	A	A	A
Case 9	35	R	1	0.53	2.48	0.95	1.39	0.34	0.61	0.68	0.17	0.36	0.09	0.24	0	7.3	-	-	-	-	-	A	A	A	A	A
Case 10	39	R	1	1.37	0.32	0.30	0.17	0.21	1.88	2.72	0.15	0.44	1.88	1.60	1.03	10.7	40	7	74	13	154	A	A	A	A	A

Case 6 has normal plasma and urine levels of 17-oxosteroids and was in remission.

n - No. of subjects in control group or no. of separate 24-hour urine collections from each patient.

* - Results expressed as the mean level of separate 24-hour urine collections.

Underlined steroid levels are elevated above the normal range.

A - Porphyrins or precursors elevated above the normal range.

TABLE 34b

17-OXOSTEROID AND PORPHYRIN LEVELS IN FEMALE PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA

Subject	Age	Attack or Remission	n	Urinary Sulphates (mg/24 hr.)				Urinary Glucuronides (mg/24 hr.)				Total Urinary	Plasma Sulphates (µg/100 ml.)				Total Plasma	Urine								
				E/A	A	E	DHA	EPI	A	E	DHA + EPI		11-OA	11-OE	11-OHA	11-OHE		17-OS	A	E	DHA	EPI	17-OS	ALA	PBG	Copro
Normal Controls	Range 40-49	Normal	10	0.87-2.31	0-0.37	0-0.53	0-0.75	0-0.39	0.11-1.41	0.28-1.78	0-0.25	0-0.21	0.04-0.85	0.03-1.45	0.01-0.45	0.6-6.7	5-42	0-8	16-85	0-20	35-127					
Case 13	41	R	1	1.32	0.21	0.21	1.50	0.71	0.95	1.32	0.59	0.42	0.65	0.86	0.63	8.0	31	11	115	8	165	A	A		A	
Case 15	41	R	1	1.26	0.46	0.29	1.41	0.44	1.95	2.75	0.16	0.33	1.17	0.70	0.82	10.5	61	9	181	17	268	A	A		A	
Case 16	42	R	1	1.43	0.27	0.32	0.61	0.26	0.73	1.09	0.04	0.19	0.65	0.70	0.32	5.1	37	12	111	15	175			A		A
Case 20	40	R	4*	1.50	0.52	0.32	0.15	0.18	1.24	2.37	0.09	0.00	0.52	0.52	0.42	6.3	37	4	93	10	144					A
Case 22	58	R	1	1.87	0.05	0.13	0.06	0.11	0.46	0.82	0.08	0.22	1.13	0.72	0.66	4.4	18	10	52	7	88					
Case 23	53	R	1	2.00	0.32	0.65	0.97	0.12	1.00	1.99	0.25	0.21	0.62	0.90	0.21	7.2	67	13	116	18	213					

Cases 11, 12, 14, 17, 18, 19 and 21 all have urinary and plasma levels of 17-oxosteroids in the normal range and were in remission

n = No. of subjects in control group or no. of separate 24-hour urine collections from each patient

* = Results expressed as the mean level of separate 24-hour urine collections.

Underlined steroid levels are elevated above the normal range.

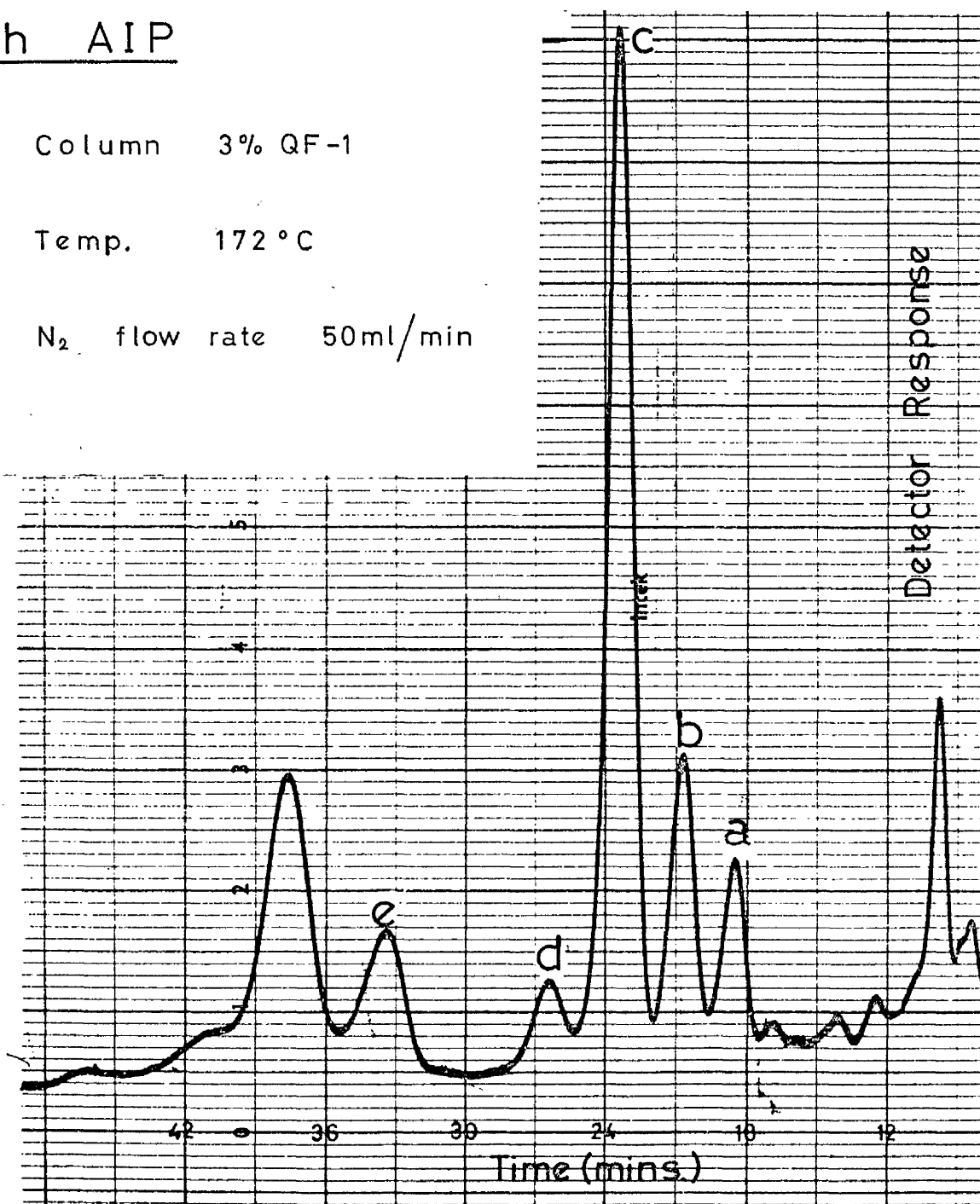
A = Porphyrins or precursors elevated above the normal range.

GLC tracing of the 17-OS sulphate
fraction from the urine of a female
with AIP

Column 3% QF-1

Temp. 172 °C

N₂ flow rate 50ml/min



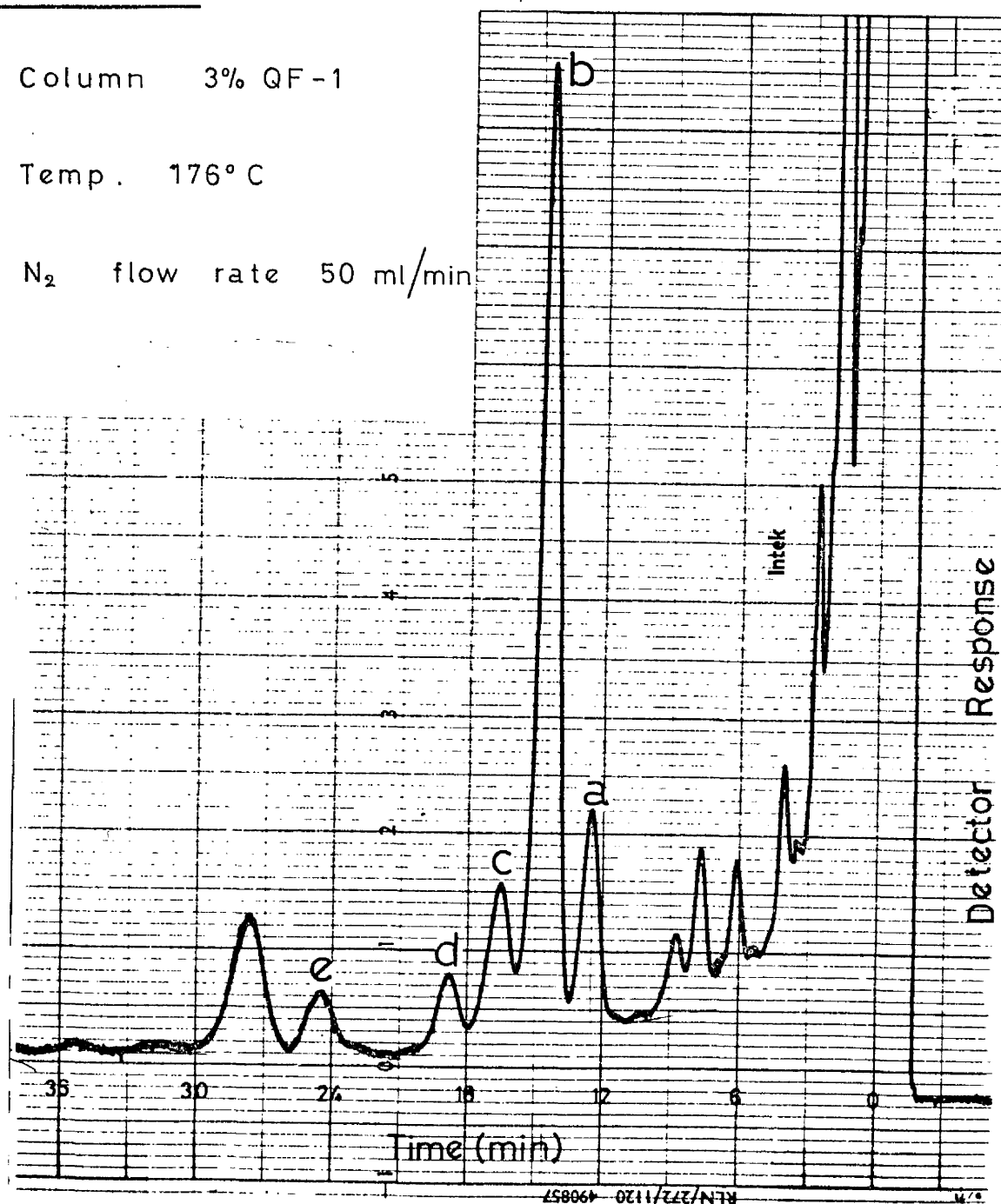
DHA (peak c) is excessively large compared to
DHA excretion in normal females.

GLC tracing of the 17-OS sulphates
from the plasma of a female patient
with AIP

Column 3% QF-1

Temp. 176°C

N₂ flow rate 50 ml/min



Large peak (b) is due to excessive quantities of aetiocholanolone sulphate in the plasma.

detected to a lesser extent in the urines of some of the patients. Apart from DHAS, elevations in the levels of AS, ES and EPIS were observed in the plasma of several patients, especially those of the 20 - 30 age group.

Use was made of Student's 't' test in the comparison of the ratio of the urinary aetiocholanolone to urinary androsterone (E/A) in the entire female AIP group with this ratio in the entire normal female control group. This E/A ratio was found to be significantly greater ($p < 0.005$) for the porphyrics than for the normals (Fig. 34a). Furthermore, the mean value of this ratio for the three patients who were suffering from an attack was not only significantly greater ($p < 0.001$) than that found for normals but was also significantly greater ($p < 0.005$) than that ratio found for the patients in remission. Similar results were found on comparing these ratios for the total number of males and females with AIP in attack with those in remission and the total normal control group (Fig. 34b).

The ages of the four females in attack ranged from twenty-three to thirty-two years, the youngest of whom was Case 3. Analyses of six complete 24-hour urine specimens and six plasma samples from this patient resulted in the detection of significant elevations of urinary ES ($p < 0.001$), DHAS ($p < 0.001$), EPIS ($p < 0.001$) and plasma AS ($p < 0.005$), ES ($p < 0.001$), DHAS ($p < 0.001$) and EPIS ($p < 0.001$). No elevations in the levels of any of the steroid glucuronides or in the total urinary excretion of 17-OS conjugates were detected. In fact, in this patient, Case 3, the urinary excretions of the glucuronide conjugates of androsterone and aetiocholanolone were significantly lower ($p < 0.001$ for both

Figure 34 a

RATIOS OF URINARY AETIOCHOLANOLONE : ANDROSTERONE IN AIP

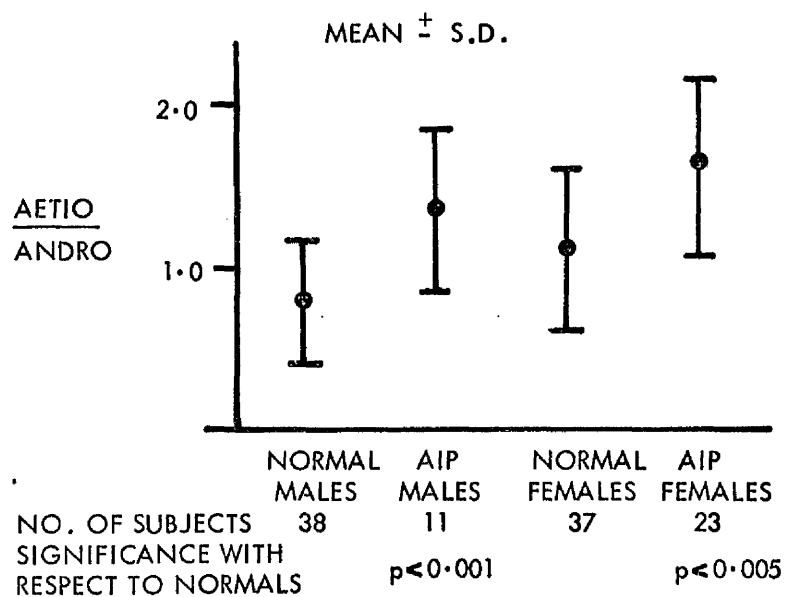
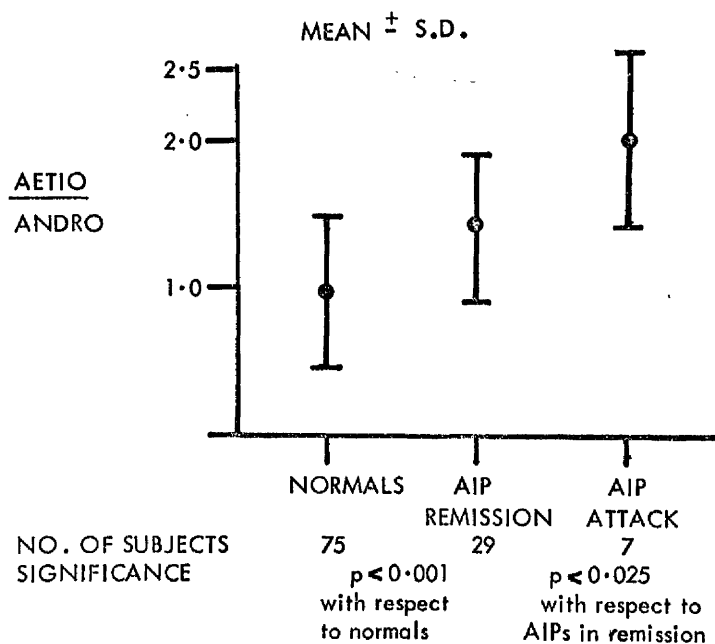


Figure 34 b

RATIO OF URINARY AETIOCHOLANOLONE : ANDROSTERONE IN AIP



steroids) than those of normal females in this 20 - 29 age group. In contrast to this, Cases 4 and 8 who were investigated during an attack, showed elevations of the total urinary 17-OS excretion and elevations in the levels of several of the glucuronide conjugates. The excretion pattern of the urinary sulphate conjugates in Case 4 was very similar to that in Case 3, both showing elevations of ES, DHAS and EPIS. In addition, Case 4 also excreted elevated amounts of aetiocholanolone glucuronide and DHA glucuronide plus epiandrosterone glucuronide. In the plasma of Case 4, unlike Case 3 where all four sulphates estimated were raised, the only elevation observed was in the concentration of DHAS. Elevations of a similar magnitude in the steroidal levels in urine and plasma were also detected in Case 8, the third patient investigated in attack. In this 32-year old female, all four urinary steroid sulphates estimated were elevated (DHAS by five times the upper limit of the normal level for that age group); in the glucuronide fraction, aetiocholanolone was elevated, and in the plasma, AS and DHAS were both elevated. Case 5 was also investigated during an attack. This young woman, aged 28 years, had a major attack of AIP five years previous to these steroidal studies. From time to time since then she has suffered from very minor exacerbations when her urine took on the characteristic port-like colour common to many porphyrics, and she experienced some mild abdominal pain. Complete 24-hour urine specimens were collected during remission and during a period when her urine was discoloured. In all the urines analysed, it was observed that the levels of DHAS, EPIS, EG and total 17-OS were greater than the upper

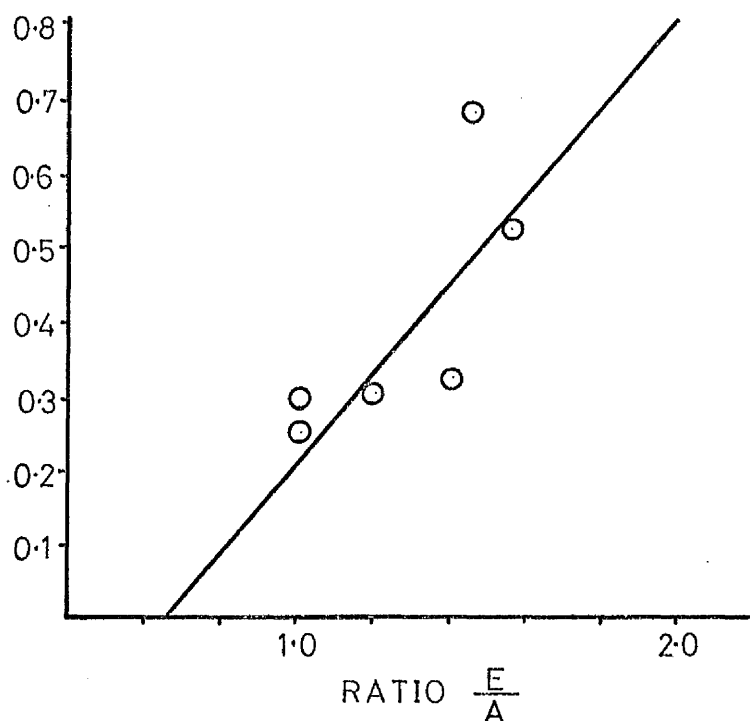
limits found in normal female subjects of that age group, but only in the discoloured urine were the levels of 11-oxo- Δ^5 -cholestanolone and 11-hydroxy- Δ^5 -cholestanolone elevated. The levels of the urinary steroids determined for the discoloured urines were generally greater than those found for the clear urines, but in both groups the elevations of DHAS, EPIS, EG and total 17-OS were very significant with $p < 0.001$. Lesser significant elevations were also observed in the levels of the conjugates of 11-OHE ($p < 0.025$), 11-OHA ($p < 0.005$), 11-OE ($p < 0.01$), 11-DA ($p < 0.05$) and DHA plus EPI ($p < 0.02$). The E/A ratio was calculated for both the discoloured and the clear urines, and it was found that the ratio was at its maximum for the discoloured urine, fell to a mean value of 1.15 for the clear urines, which is still significantly greater ($p < 0.001$) than that found for normal females in that same age group. In the discoloured urines, excessive quantities of ALA, PBG, uroporphyrin and coproporphyrin were found, and it was these urines which had the highest values of E/A ratio. In fact, fairly good linear correlations were obtained between the values of the E/A ratio and the levels of the porphyrins and precursors in the individual urines. As the value for the ratio fell, the corresponding levels of ALA, uroporphyrin, coproporphyrin and PBG decreased with correlation coefficients (r) of 0.78, 0.84, 0.81 and 0.75 respectively (Fig. 35).

These four patients, who were in attack, all excreted excessive quantities of uroporphyrin, coproporphyrin, ALA and

The correlation between the urinary ratio of E/A and the urinary ALA excretion in a female patient with AIP

Case 5

ALA
mg %

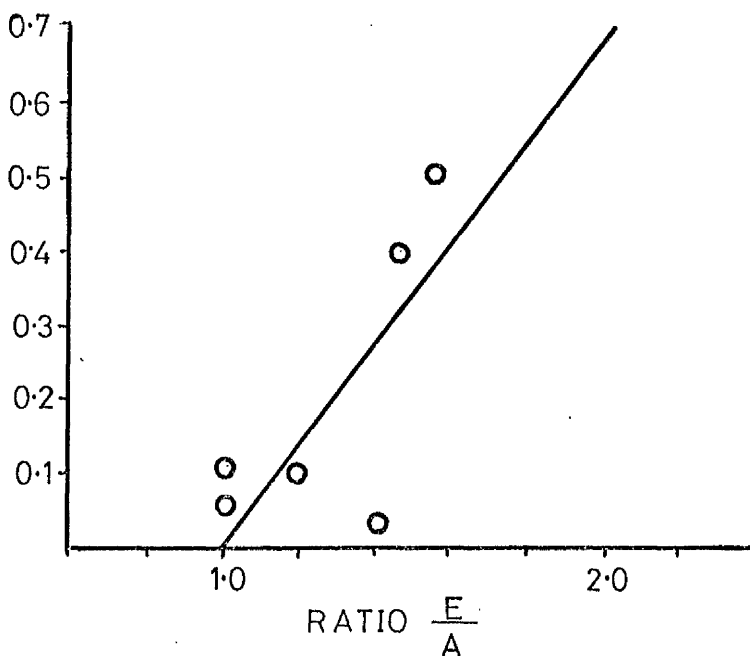


$$y = 0.603x - 0.386 \quad (r = 0.78)$$

The correlation between the urinary ratio of E/A and the urinary PBG excretion in a female patient with AIP

Case 5

PBG
mg %



$$y = 0.682x - 0.682 \quad (r = 0.75)$$

PBG in their urine.

These steroid abnormalities were not confined to those patients suffering from an attack but were also found in patients in various stages of remission. An example of this is Case 1, who is a young woman aged 26 years. She was admitted to hospital in 1966 with a severe attack of AIP. The attack had apparently been precipitated after her attendance at a party where she had taken alcohol for the first time, but it later transpired that she had also taken several barbiturate tablets for symptomatic relief early in her illness. She made a gradual recovery from this attack and six weeks later was discharged from hospital, but was readmitted two years later with another acute attack with abdominal pain, vomiting and constipation. Once again, she made a gradual recovery and since then she has remained entirely symptom free. Levels of her urinary 17-OS conjugates were reported by Goldberg et al. (1969) eight months after her last attack. Significant elevations of EG, DHAG plus EPIG, DHAS and EPIS were found. Two years later, the urinary levels along with the plasma levels were re-examined by the author. During this period, no attacks are known to have occurred. The urinary levels of ES, DHAS, EPIS and total 17-OS excretion were found to be elevated above the normal limit. No elevations were observed in the glucuronide fraction of the urine. Investigation of the plasma of this subject revealed elevations in the concentrations of all four 17-OS sulphates. Particularly striking was the massive increase of aetiocholanolone

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sulphate which usually occurs in normals at concentrations of 0 - 12 $\mu\text{g}/100$ ml of plasma, but in this patient had increased to 435 $\mu\text{g}/100$ ml of plasma. Although this patient had been symptom-free for the last four years, she still excreted excessive quantities of ALA, PBG, coproporphyrin and uroporphyrin in the urine.

Case 2 is an identical twin of Case 1, and was also admitted to hospital in 1966 with a similar but less severe attack. She also had consumed alcohol at the same party for the first time and, like her sister, had also taken barbiturate for symptomatic relief early in her illness. She made a gradual recovery and since then has not experienced any further symptoms. Goldberg et al. (1969) reported the levels in the urine of her 17-OS conjugates, two-and-a-half years after her last attack. They found significant elevations in the levels of EG, DHAG plus EPIG and EPIS, but the levels were not as high as those levels found in her sister. Examination of a urinary specimen from this patient in 1971 by the author resulted in the detection of elevations in the levels of aetiocholanolone, DHA and epiandrosterone in the urinary sulphate fraction but no elevations were observed in the glucuronide fraction. In fact, the glucuronide levels were noted to be lower than the levels of the normal subjects of this age group. The total urinary excretion of the 17-OS conjugates was also found to be just below the normal lower limit for the 20 - 29 age group. Analysis of this patient's plasma revealed increased levels of all the 17-OS sulphates but not to such high levels as those of her twin sister.

Those lower levels of steroids in this patient compared to her sister were also associated with a clinically less severe involvement of the disease and a lower level of urinary excretion of porphyrins and precursors, although these levels are still much greater than those of normal subjects.

One latent acute intermittent porphyric patient, Case 21, was studied. This patient was a 52-year old woman who suffered from hypertension. Elevated levels of coproporphyrin and uroporphyrin were found in her urine but the ALA and PBG levels were normal. No abnormalities were detected in the 17-OS excretion in the urine of this patient.

All the remaining patients with AIP were in various stages of remission ranging from two years to twenty years, and presented a wide range of steroid abnormalities. Two of these patients, Cases 10 and 13, excreted excessive quantities of all four porphyrins and precursors. Both also excreted excessive quantities of total 17-OS conjugates in the urine. In Case 10, elevations were not detected in the urinary sulphate fraction or in the plasma but were confined to the glucuronide fraction, where aetiocholanolone and all four 11-oxy-17-OS were found to be raised. Similar elevations of the 11-oxy-17-OS were observed in Case 13. This patient also had increased urinary excretion of both DHAS, DHAG plus EPIG and increased concentration of DHAS in the plasma.

Eight patients out of the entire group did not display any elevations of the urinary excretion of the 17-OS conjugates or of the concentrations of the 17-OS sulphates in the plasma.

All of these patients, with the exception of one who was a latent AIP, were in remission. Of these eight, three patients (Cases 6, 18 and 19) excreted elevated amounts of ALA and PBG, two (Cases 14 and 21) excreted elevated quantities of urinary porphyrins and three (Cases 11, 12 and 17) excreted normal amounts of uroporphyrin, coproporphyrin, ALA and PBG.

Seven patients from the entire group had normal excretions of the porphyrins and precursors and, as just mentioned, three of them had normal steroid levels. It might be noted that the only abnormality observed in the steroid levels of Case 22 was the slight elevation beyond the upper limit of normal of the 11-oxo-aetiocholanolone glucuronide. Of the three remaining cases with normal porphyrin and precursor excretion in the urine, Case 5, which has been discussed previously, had significant elevations of urinary ES, DHAS, EPIS, EG and DHAG plus EPIG (the plasma was not analysed during remission); Case 20 had increases in the levels of urinary AS and EG with no increases in the plasma, and Case 23 had elevations in the levels of ES, DHAS and EG in the urine and AS and DHAS in the plasma.

C. STEROIDAL STUDIES IN MALES WITH AIP.

A total of eleven males with AIP were investigated. Of these eleven, three were examined during an attack, and the remaining at various stages of remission (Table 35). Only one patient, Case 34, from the entire group revealed normal levels of porphyrins and precursors in the urine. This subject was seventy-nine years old and had been in remission for over twenty years. The steroid levels observed in the urine and plasma of this subject were also normal. One other subject, Case 31, from this group had normal steroid levels. This patient also has been in remission for some time but, unlike Case 34, excessive quantities of ALA, PBG and coproporphyrin were detected in the urine.

The most striking steroid abnormality detected in the urine was the increased excretion of aetiocholanolone sulphate and glucuronide, and DHA sulphate. In the plasma, DHAS was the steroid that was most consistently raised in this group of patients. These excessively high levels of DHAS in the urine and plasma are represented by the large peak (c) in the typical g.l.c. traces of male patients with AIP depicted in Figs. 36 and 37. These abnormalities are similar to those observed in the female AIP group. As with the female group, the mean E/A ratio for the male AIP patients was significantly greater ($p < 0.001$) than that obtained for the entire male control group (Fig. 34a).

In this study, three male patients were investigated during an attack of AIP. Case 24 was a young man aged thirty,

TABLE 35

17-OXOSTEROID AND PORPHYRIN LEVELS IN MALE PATIENTS WITH ACUTE INTERMITTENT PORPHYRIA

Subject	Age	Attack or Remission	n	E/A	Urinary Sulphates (mg/24 hr.)					Urinary Glucuronides (mg/24 hr.)					Total Urinary	Plasma Sulphates (µg/100 ml.)				Total Plasma	Urine										
					A	E	DHA	EPI	A	E	DHA + EPI	11-OA	11-OE	11-OHA		11-OHE	A	E	DHA		EPI	ALA	PBG	Copro	Uro						
Normal Controls	Range 30-39	Normal	10	0.47-1.78	0.35-2.08	0.06-0.68	0.19-4.15	0.11-0.82	0.11-10.10	0.47-5.66	0.14-1.82	0.08-0.82	0-1.51	0-2.80	0-1.54	3.35-25.18	18-88	3-15	18-153	8-24	54-259										
Case 24	30	A	4*	2.27	1.24	1.38	3.79	0.64	1.72	5.33	1.93	0.69	0.93	1.02	0.89	19.6	-	-	-	-	-	A	A	A	A	A	A	A	A	A	A
Case 24	30	R	1	1.52	1.25	0.63	2.26	0.15	1.61	3.73	0.23	0.0	0.58	0.66	0.37	11.5	-	-	-	-	-	A	A	A	A	A	A	A	A	A	A
Case 26	37	A	11*	2.22	0.76	0.31	5.86	0.32	2.54	7.15	2.63	0.70	1.02	0.53	0.58	22.4	-	-	-	-	-	A	A	A	A	A	A	A	A	A	A
Case 26	37	R	10*	2.12	0.60	0.44	3.46	0.68	2.38	5.88	1.14	0.34	0.66	0.37	0.61	16.5	-	-	-	-	-	A	A	A	A	A	A	A	A	A	A
Normal Controls	Range 40-49	Normal	8	0.56-1.89	0.05-0.85	0.03-0.36	0.09-2.27	0.02-0.29	0.11-3.70	0.38-2.20	0.06-0.81	0.06-0.85	0-1.03	0.02-2.45	0.02-1.10	3.03-12.00	20-59	0-11	26-108	5-19	62-179										
Case 27	42	A	1	0.74	3.15	1.87	5.98	1.24	1.08	1.24	0.89	0.13	0.99	0.74	0.55	17.9	91	10	220	27	348	A	A								
Case 28	45	R	1	1.64	0.56	0.78	1.30	0.28	2.48	4.19	0.33	0.14	1.44	1.23	0.80	13.5	87	9	204	20	320	A	A	A	A	A	A	A	A	A	A
Case 29	46	R	1	0.96	0.48	0.24	0.42	0.12	1.59	1.75	0.15	0.12	1.28	0.61	0.97	7.7	80	4	143	18	245	A	A	A	A	A	A	A	A	A	A
Case 30	45	R	1	0.76	0.52	0.34	0.23	0.22	2.20	1.82	0.10	0.25	1.50	1.42	0.67	9.2	88	7	94	22	211	A	A	A	A	A	A	A	A	A	A
Case 32	48	R	3*	1.19	0.29	0.20	0.22	0.39	2.28	2.85	0.18	0.08	0.49	0.56	0.48	8.0	-	-	-	-	-	A	A								
Normal Controls	Range 58-63	Normal	2	0.37-1.57	0.08-0.60	0.06-0.16	0.02-0.44	0.09-0.28	0.22-0.96	0-0.41	0-0.08	0-0.09	0.10-0.23	0.02-1.23	0-0.55	1.30-5.03	5-32	0-4	0-76	8-14	14-122										
Case 33	62	R	1	1.15	0.18	0.09	0.09	0.09	1.24	1.55	0.10	0.17	1.08	0.69	0.44	5.7	28	7	36	10	81	A	A								

Cases 25, 31 and 34 all have steroids in the normal range and were in remission.

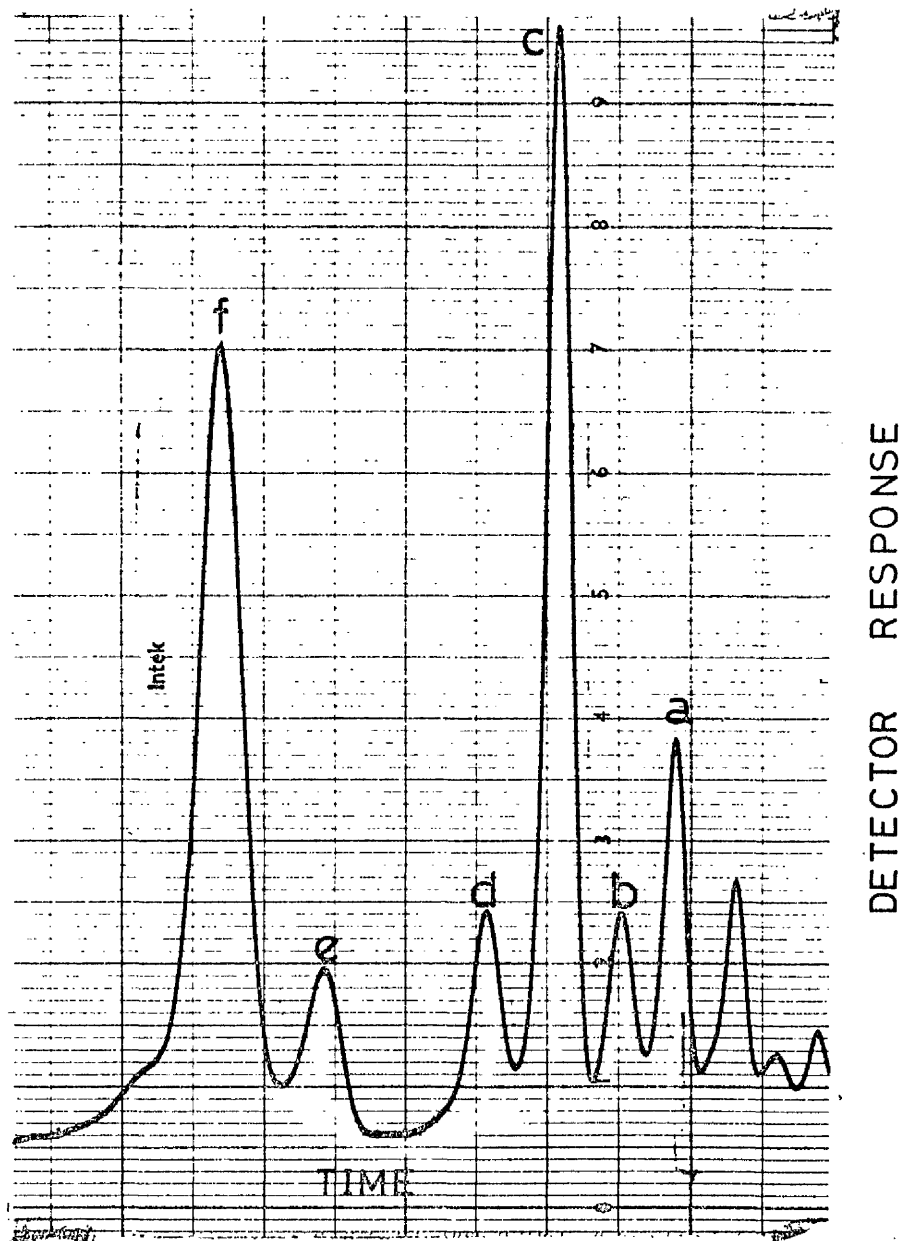
n - No. of subjects in control group or no. of separate 24-hour urine collections from each patient.

* - Results expressed as the MEAN level of separate 24-hour urine collections.

Underlined steroid results are elevated above the normal range.

A - Porphyrins or precursors elevated above the normal range.

GLC tracing of the 17-oxosteroid sulphates from
the urine of a male patient with AIP

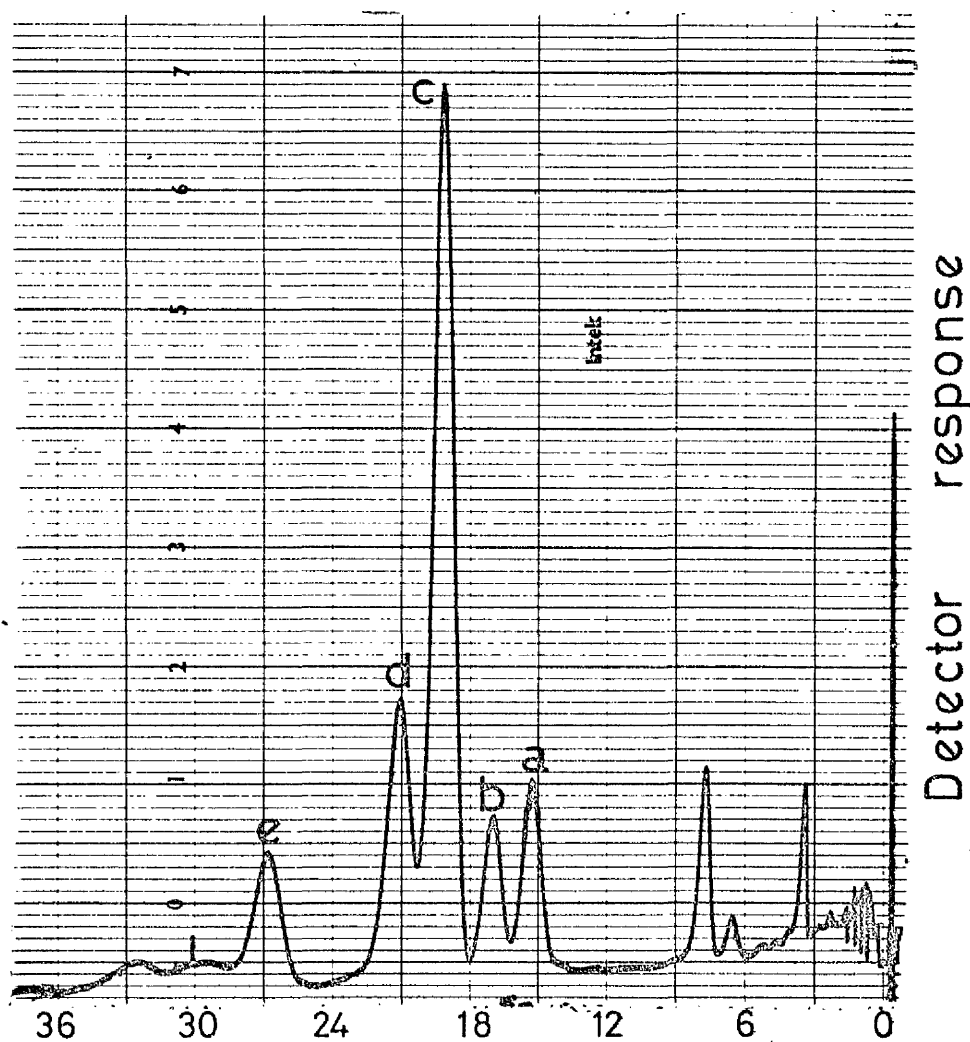


Excessive excretion of DHA sulphate is represented by the large peak (c)

Figure 37

GLC tracing of the 17-oxosteroid sulphates
from the plasma of a male patient with

AIP



Excessive quantities of the sulphate conjugates of DHA and aetiocholanolone are represented by the peaks 'c' and 'b' respectively.

who had suffered from several acute attacks. During this last attack, excessive excretions of ALA, PBG, uroporphyrin and coproporphyrin were detected in his urine. The concentration of uroporphyrin reached a value of 2578 $\mu\text{g/l}$, and coproporphyrin a value of 452 $\mu\text{g/l}$ during this attack. The normal values for the concentration of uroporphyrin and coproporphyrin in urine are $1.3 \pm 0.8 \mu\text{g/l}$ (mean \pm S.D.) and $53.7 \pm 44.8 \mu\text{g/l}$, respectively (Moore et al., 1972a). Significant elevations in the urinary levels of EG ($p < 0.001$), ES ($p < 0.001$) and DHAG plus EPIG ($p < 0.001$) were recorded but the total urinary 17-OS level was within the normal range of that age group. Calculation of the urinary E/A ratio produced a value of 2.27 ± 0.28 , which is significantly greater ($p < 0.001$) than that calculated for the normals of the same age group. One year after this last attack, another 24-hour urine specimen from this patient was examined. This analysis revealed that the total 17-OS level in the urine had fallen from $19.6 \pm 1.6 \text{ mg/24 h}$ during the previous year's attack to a value of 11.5 mg/24 h ; no abnormally high steroid conjugate levels were observed in the urine, and the urinary E/A ratio had decreased to 1.52, but it must be noted that this value is still greater than the mean for the control group. Determination of the ALA, PBG, uroporphyrin and coproporphyrin content of the urine revealed excessive amounts of all four compounds, but these levels were lower than those previously recorded during the previous year's attack.

The second patient to be examined in attack was Case 27,

a 42-year old man who had suffered several mild attacks of AIP. Only one complete 24-hour urine specimen and one plasma specimen were obtained from this patient, and steroidal analyses revealed large increases of all the urinary sulphate conjugates, a small increase in the urinary level of DHAG plus EPIG, an elevation in the total urinary 17-OS excretion and, finally, an increase in the concentrations of DHAS, AS and EPIS in the plasma. These steroid abnormalities were accompanied by increased excretion of ALA, PBG and uroporphyrin in the urine.

Case 26 was the third patient who was investigated during an attack. This subject, who is aged thirty-seven years, has suffered from repeated acute attacks, principally of abdominal pain, but on occasion also with weakness and paralysis, since 1950. In the six years since 1966, he has been admitted to hospital on numerous occasions, on each of which he has had tachycardia and mild hypertension, and there has been a striking rise of ALA and PBG in the urine. In a previous investigation of this subject, Goldberg et al. (1969) reported that the urinary DHAS of this patient was significantly raised ($p < 0.01$) during an acute attack. Further investigations of the urinary steroid levels of this patient by the author are presented in Table 35. Analyses of several 24-hour urine specimens during another more severe attack confirmed the previous findings by Goldberg et al. (1969) of abnormally high levels of some of the urinary steroids in this patient. In a series of eleven 24-hour urine specimens obtained during an attack, significant elevations were found in the levels of DHAS ($p < 0.001$), of DHAG plus EPIG ($p < 0.001$) and of EG

($p < 0.005$). The total urinary 17-OS excretion during the attack was also found to be significantly elevated ($p < 0.05$). The E/A ratio was calculated for these urines, and it too was found to be significantly greater ($p < 0.001$) than that calculated for the normals of the same age group. Analyses were also carried out on 24-hour urine specimens of this patient when he was in remission. They revealed levels of the steroids whose mean excretion values were not greater than the upper limits of normal, but the mean levels of DHAS and EG still remained significantly greater ($p < 0.05$ and $p < 0.02$, respectively) than those of normals of the same age group. There was no significant change in the value of the E/A ratio when the patient was in remission.

Since the main precursors of these excretory metabolites in the urine are secreted by the adrenal cortex and, in particular, since DHAS has been shown to be quantitatively one of the most important products secreted by the adrenal cortex (Baulieu et al., 1965), it was decided to study the effects of dexamethasone use to suppress adrenal function in this AIP patient. Administration of dexamethasone suppresses ACTH secretion and, thus, ACTH-dependent steroid production by the adrenal cortex. In particular, this will lead to the suppression of the production of DHA, and DHAS, which is the principal C_{19} steroid synthesised by the human adrenal cortex (Wieland et al., 1963), being secreted in amounts of the order of 10 - 15 mg/24 h and which appears to be the major source of the 11-deoxy-17-OS found in the urine. Following dexamethasone administration to this AIP patient, highly

significant reductions in the levels of all the urinary 17-OS conjugates, except aetiocholanolone sulphate, were observed. In addition, concurrent reductions in the levels of the porphyrins and precursors in the urine were also observed (Table 36). These findings were accompanied by a noticeable improvement in the clinical condition of the patient. The possible relationship between the administration of dexamethasone to this AIP patient and the significant fall in the levels of the urinary porphyrins and precursors will be discussed in the final section of this thesis.

Table 36

THE EFFECT OF DEXAMETHASONE TREATMENT ON A MALE PATIENT
WITH ACUTE INTERMITTENT PORPHYRIA

(each figure is the mean \pm S.D. of 22 individual measurements)

	Urinary levels (mg/24 h)		
1. Porphyrins & Precursors	Before treatment	Dexamethasone treatment	Significance (p<)
Uroporphyrin	3.93 \pm 1.19	0.53 \pm 0.28	0.001
Coproporphyrin	0.49 \pm 0.28	0.26 \pm 0.17	0.02
δ -aminolaevulinic acid	61.14 \pm 23.24	29.54 \pm 13.81	0.001
Porphobilinogen	126.30 \pm 27.86	80.52 \pm 26.89	0.001
2. <u>Steroids</u>			
Total 17-oxosteroids	11.89 \pm 3.91	7.66 \pm 1.73	0.001
<u>Sulphates</u>			
Dehydroepiandrosterone	4.44 \pm 2.48	0.32 \pm 0.21	0.001
Androsterone	0.63 \pm 0.18	0.34 \pm 0.13	0.001
Aetiocholanolone	0.39 \pm 0.14	0.34 \pm 0.14	N.S.
Epiandrosterone	0.51 \pm 0.25	0.09 \pm 0.09	0.001
<u>Glucuronides</u>			
Dehydroepiandrosterone + Epiandrosterone	1.94 \pm 1.00	0.14 \pm 0.12	0.001
Androsterone	2.20 \pm 1.02	0.75 \pm 0.34	0.001
Aetiocholanolone	5.76 \pm 1.81	2.47 \pm 1.71	0.001

D. STEROIDAL STUDIES ON PATIENTS WITH HEREDITARY
COPROPORPHYRIA.

Hereditary coproporphyria (HCP), like acute intermittent porphyria, is a hepatic porphyria which is characterized from the other hepatic porphyrias by the excessive excretion of coproporphyrin in the faeces and the urine at all times, accompanied by an elevation in porphyrin precursor excretion in acute attack.

In this study, five patients, one male and four females, suffering from HCP, were investigated. The results of estimation of urinary 17-OS conjugates are presented in Table 37. Three of these patients were suffering from an acute attack at the time of the investigation. The only male HCP, Case 35, to be studied was aged forty-eight, and he had originally been investigated in 1966 because his sister was severely ill with HCP. He had a history of nervousness for a number of years, and rather large quantities of alcohol had been consumed to relieve the symptoms. No history of abdominal pain, vomiting, constipation or tachycardia was apparent. No discolouration had ever been observed in his urine, but analysis of his urine and stool revealed grossly elevated levels of coproporphyrin with normal urinary levels of ALA, PBG and uroporphyrin. None of the steroid conjugates measured in the urine were elevated above normal, but the E/A ratio was greater than the mean normal ratio for that age group.

The youngest patient investigated in this study was a

TABLE 37

17-OXOSTEROID AND PORPHYRIN LEVELS IN PATIENTS WITH HEREDITARY COPROPORPHYRIA

Subject	Age	Attack or Remission	n	Urinary Ratio		Urinary Sulphates (mg/24 hr.)						Urinary Glucuronides (mg/24 hr.)						Total Urinary	Urine			
				E/A	A	E	DHA	EPI	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	17-O5	ALA		PBG	Copro	Uro	
Normal ♂ Controls	Range 40-49	Normal	8	0.56-1.89	0.05-0.85	0.03-0.36	0.09-2.27	0.02-0.29	0.11-3.70	0.38-2.20	0.06-0.81	0.06-0.85	0-1.03	0.02-2.45	0.02-1.10	3.0-12.0						
35 ♂	47	R	1	1.33	0.25	0.17	1.46	0.23	1.05	1.55	0.42	0.10	0.52	0.59	0.34	6.7			Λ			
Normal ♀ Controls	Range 20-29	Normal	15	0.47-1.35	0-0.89	0.03-0.55	0-0.78	0-0.32	1.51-4.43	1.46-4.00	0-0.47	0.07-0.27	0.15-1.54	0.29-1.38	0-1.00	5.5-10.9						
Case 36 ♀	20	R	2	0.76	0.07	0.22	0.14	0.16	3.15	2.22	0	2.12	0.25	0	0	8.3			Λ	Λ		
Case 36 ♀	20	A	5*	2.17	0.25	0.65	0.03	0.97	0.63	1.20	0.19	0.68	0.43	0.11	0.36	5.5	Λ	Λ	Λ	Λ		
Case 37 ♀	28	R	1	1.11	0.03	0.06	0.07	0.05	0.61	0.65	0.06	0.07	0.34	0.50	0	2.5			Λ	Λ		
Case 38 ♀	25	A	7*	2.06	0.04	0.09	0.10	0.42	1.99	4.10	0.23	0.10	0.73	0.42	0.07	8.3	Λ	Λ	Λ	Λ		
Normal ♀ Controls	Range 30-39	Normal	11	0.63-2.16	0.02-0.96	0.06-0.38	0.01-0.80	0.04-0.33	0.16-1.90	0.30-2.41	0-0.32	0-0.27	0.20-1.04	0.02-1.38	0-0.75	1.6-7.5						
Case 39 ♀	30	A	1	2.38	0.04	0.46	0.06	0.02	0.53	0.90	0.23	0	1.81	0	12.46	16.5	Λ	Λ	Λ	Λ		

n - No. of subjects in control group or no. of separate 24-hour urine collections from each patient.

* - Results expressed as the MEAN values of separate 24-hour collections.

Underlined steroid levels are elevated above the normal range.

A - Porphyrins or precursors elevated above the normal range.

twenty-year old female - Case 36. Her first attack had occurred at the age of seventeen, when she was admitted to hospital suffering from colicky, abdominal pain and diarrhoea. No family history of the disease was apparent and, previous to this attack, the patient had been healthy. Physical examination revealed considerable tenderness in the right iliac fossa, and appendicectomy was carried out using thiopentone sodium to induce anaesthesia. The appendix was of normal appearance. Post-operatively, she vomited considerably and developed a mild pyrexia. At this stage, it was noted that she was two months pregnant. Four weeks after the appendicectomy, she became slightly icteric and, three weeks later she developed a bullous eruption on the light exposed parts of her body. Analyses of her urine and faeces at this point revealed the excretion of massive amounts of coproporphyrin. These eruptions on the skin subsided after one week and, thereafter, she remained comparatively well until she was delivered of a still-born child. Her good health continued throughout her second pregnancy but, one week after the delivery of a normal healthy child, she became acutely ill with abdominal pain and vomiting. Her blood pressure rose to 220/130 mm Hg and she suffered several epileptic seizures. Determinations of the 17-oxosteroid levels in two twenty-four hour urine specimens prior to the patient's acute attack revealed an elevation in the excretion of the 11-OA glucuronide but not in any of the 11-deoxy-17-OS. In these two urines, the concentrations of coproporphyrin and uroporphyrin were raised, but those of ALA

and PBG were not. During the acute episode, five complete 24-hour urine specimens were analysed. The level of the 11-OAG was found to remain elevated, but not at such high levels as was observed in the two urines collected prior to the attack. Significant elevations in the urinary levels of ES and EPIS ($p < 0.025$ for both) were observed. These were accompanied by the excretion in the urine of large amounts of ALA and PBG. During the attack, the urinary ratio of E/A was significantly greater ($p < 0.001$) than that found for the urines collected before the commencement of the acute episode, and some positive correlation was found between the urinary E/A ratio and the urinary level of ALA ($r = 0.82$) and between the urinary E/A ratio and the urinary level of PBG ($r = 0.78$) (Fig. 38). These lines have the equations $y = 18.40x - 2.00$ and $y = 27.60x - 5.18$, respectively.

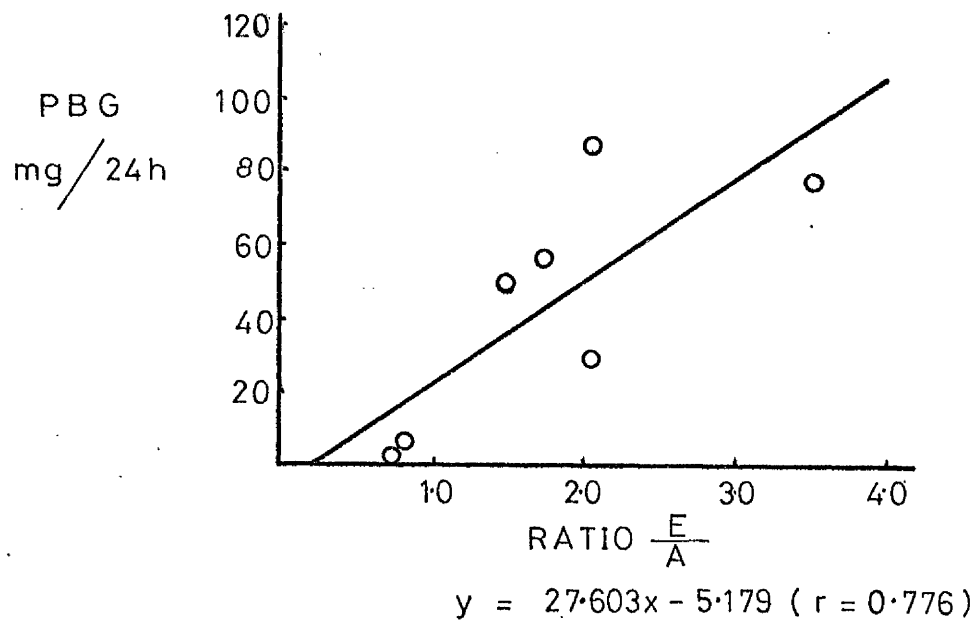
The duration of the attack was approximately ten days and, thereafter, rapid improvement in her condition was observed. Since that time, there has been no reappearance of any of the symptoms. Porphyrin analyses of her family revealed three examples of the disorder in latent form.

Case 39, a thirty-year old housewife, was also investigated during a period of attack. The first symptoms, persistent lower abdominal pain, anorexia, constipation and symptoms of cystitis, occurred after hysterectomy and appendicectomy. She had difficulty in sleeping and became increasingly agitated and depressed. This patient had a history of nervousness and

Figure 38

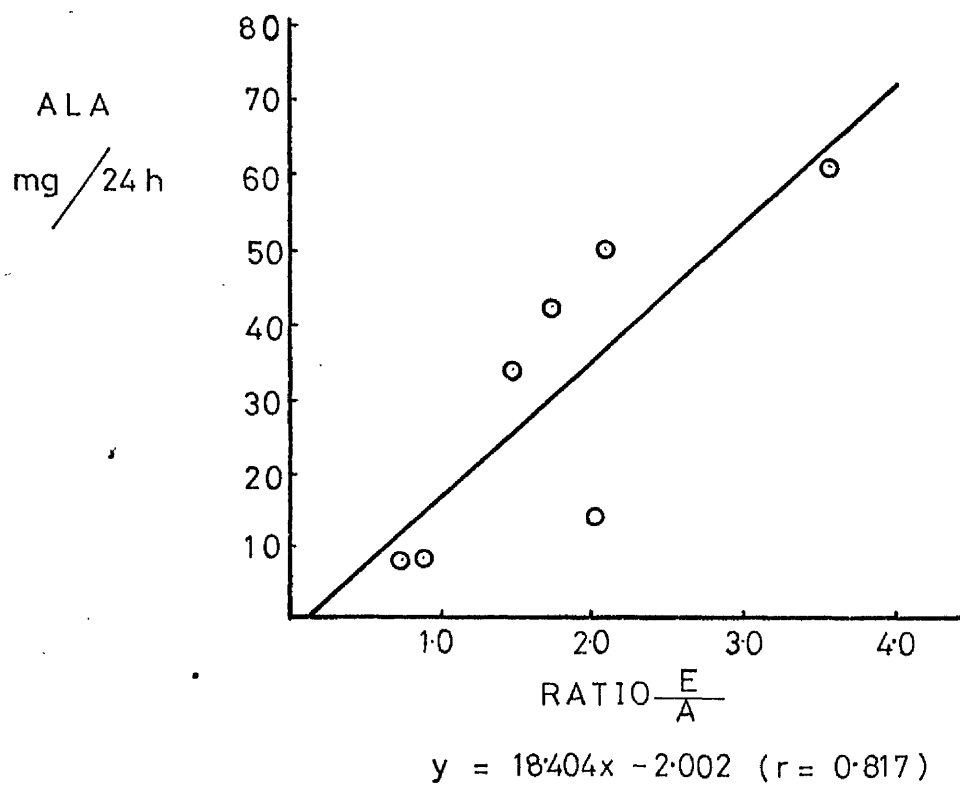
The correlation between the urinary ratio of E/A and the urinary

PBG excretion in a female HCP patient Case 36



The correlation between the urinary ratio of E/A and the

urinary ALA excretion in Case 36



depression, and anorexia nervosa had been diagnosed at the age of 21 years. Following hysterectomy, post-operative management included treatment with a variety of drugs including butobarbitone, promethazine, papaveretium and several antibiotics. The patient's condition worsened with time, and a diagnosis of agitated depression with hysterical features was made. She was further treated with amylobarbitone and imipramine, but her condition deteriorated with the appearance of confusion and paranoia. Her urine had been noted to be dark during her surgical admission but it was not until much later, when excessive amounts of PBG were found in the urine, that the significance of this finding was appreciated. The analyses of her urine and faeces resulted in a diagnosis of an acute attack of hereditary coproporphyria being made. Duplicate steroidal determinations in one of the 24-hour specimens obtained during an attack revealed small elevations in the levels of ES and 11-OEG and a massive elevation in the level of the 11-hydroxy-aetiocholanolone glucuronide. The value estimated for the urinary excretion of this steroid was 12.5 mg/24 h, which is more than twelve times the maximum value estimated for normal subjects of the same age group. In contrast to this great elevation of the levels of the 11-oxygenated aetiocholanolone glucuronides, there was no 11-oxygenated androsterone glucuronides detectable in the patient's urine.

Having established the diagnosis of hepatic porphyria, therapy with barbiturates and other drugs was immediately ceased, and treatment with chlorpromazine instituted. Within 24 hours,

the persistent abdominal pain had disappeared but her mental state remained abnormal for an additional month. Two months later, after a gradual improvement of her mental health, she was discharged from hospital. Since then, investigation of other members of her family have revealed two latent hereditary coproporphyrrias, her mother and a sister, who excreted excessive amounts of coproporphyrin in the urine and faeces.

The third patient, Case 38, who was investigated during an attack, was another young lady aged 25 years. Seven complete 24-hour urinary specimens were obtained from this patient during the acute episode. Estimations of the porphyrins and precursors in these urines revealed highly elevated levels of coproporphyrin, uroporphyrin, ALA and PBG in all seven urines analysed. When the fractionated urinary 17-oxosteroids were determined in these urines, it was found that the levels of EPIS ($p < 0.02$) and EG ($p < 0.001$) were significantly elevated. The total urinary 17-OS excretion was within the limits of the normal females of that same age group. Calculation of the urinary E/A ratio for this patient gave a value of 2.0 ± 0.2 , which is significantly greater ($p < 0.001$) than that for normals (Fig. 39). As with Case 36, there was some degree of linear correlation between the fall of the levels of ALA and PBG in the urine and the fall of the urinary E/A ratio (Fig. 40). The relationship between the urinary level of ALA and the urinary ratio of E/A has a coefficient of correlation $r = 0.81$ and an equation $y = 27.47x - 34.72$. Similarly, the relationship

Figure 39

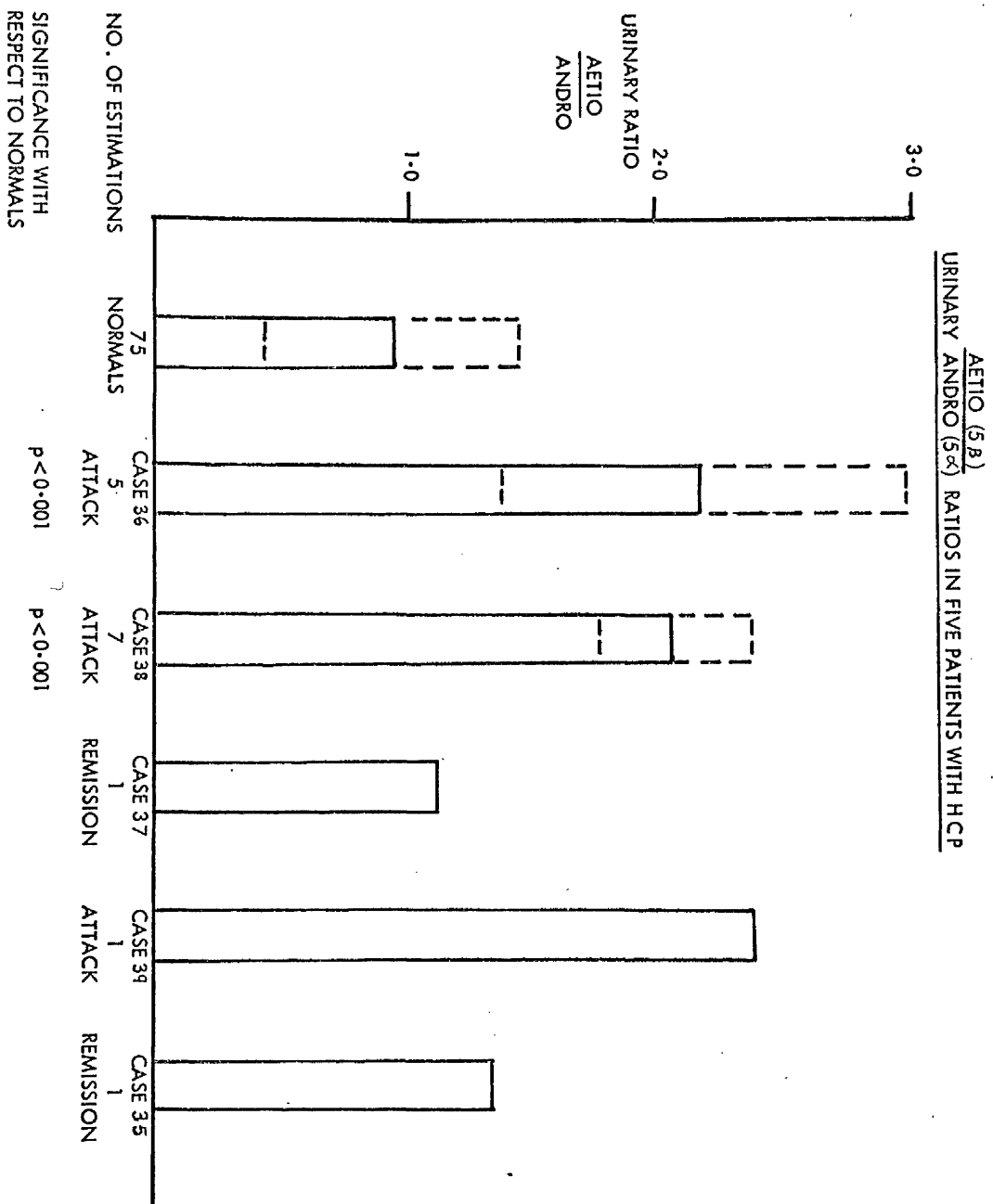
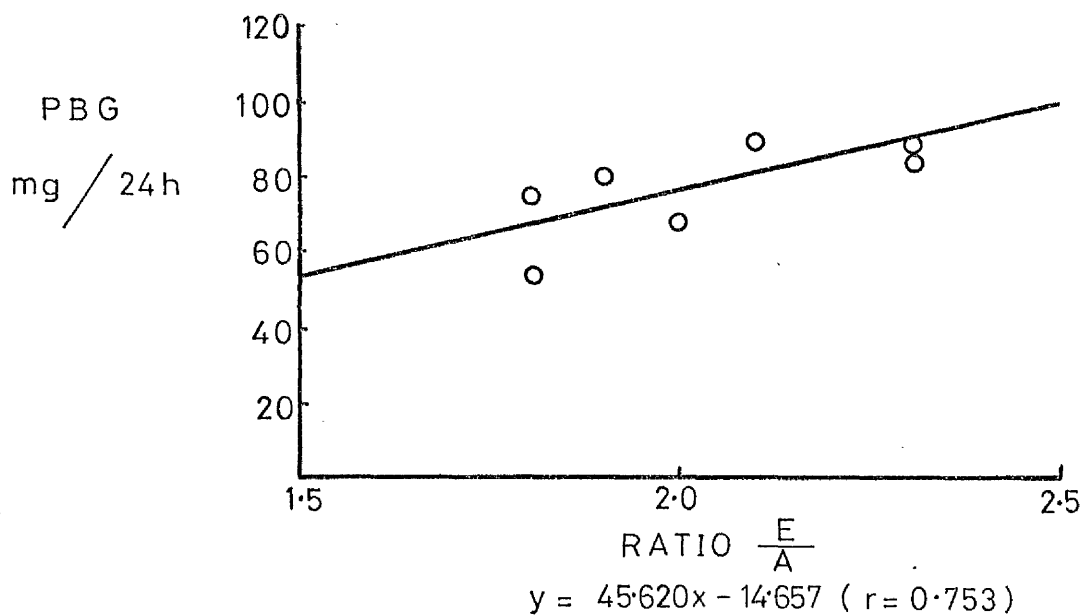
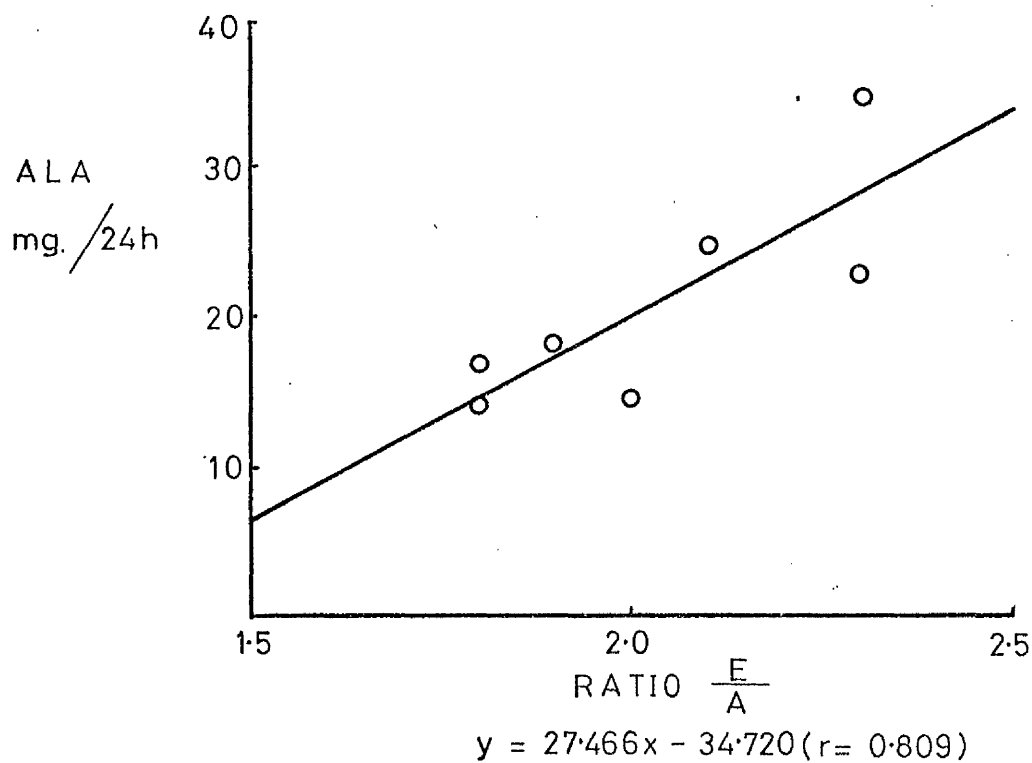


Figure 40

The correlation between the urinary ratio of E/A and the urinary
PBG excretion in a female HCP patient, Case 38



The correlation between the urinary ratio of E/A and the urinary
ALA excretion in Case 38



between the urinary level of PBG and the E/A ratio gave a coefficient of correlation $r = 0.75$ and an equation $y = 45.62x - 14.66$.

The final patient to be investigated in this group of HCP subjects was Case 37, a 28-year old housewife who first presented in 1966 during her second pregnancy with a urinary tract infection. After treatment with sulphonamides, her urine was noted to be dark and was found to contain increased amounts of PBG. Her only other symptom was depression of 2 years duration. Quantitative porphyrin analysis showed large amounts of coproporphyrin in both urine and faeces. The pregnancy ended with a neonatal death, the cause of which was uncertain. During the early stages of her third pregnancy, this patient once again presented with a further attack of depression, but this settled quickly and pregnancy culminated with the birth of a normal baby. Since that time, no further bouts of depression or any other symptoms of porphyria have been experienced. The urine which was used for steroid estimations was obtained fourteen months after the last attack of depression. Elevations in the levels of the fractionated 17-oxosteroids were not observed. Quantitative porphyrin analysis of the same urine showed increased levels of uroporphyrin and coproporphyrin but not of ALA and PBG. No evidence of hereditary coproporphyrin has been found in other members of her family.

E. STEROIDAL STUDIES ON PATIENTS WITH VARIEGATE PORPHYRIA.

Variegate porphyria is a hereditary hepatic porphyria, biochemically characterised by highly elevated amounts of urinary uroporphyrin and coproporphyrin, faecal proto-porphyrin, coproporphyrin and X-porphyrin, and variable elevation of ALA and PBG in the urine. No conclusions as to whether abnormalities in the steroid levels might exist in VP could be reached as only two patients were investigated in this study. One was a fifty-three year old woman who had been in remission for eight years. She had suffered the last attack in 1962 after hysterectomy. Quantitative analysis of her urine revealed normal excretion of porphyrins and precursors, and also normal levels of 17-OS conjugates. The other was a twenty-four year old male who had been in remission for three years. Although urinary levels of ALA, PBG and porphyrins were elevated, no similar abnormalities were observed in the 17-OS conjugate levels, either in the plasma or the urine (Table 38).

17-OXOSTEROID AND PORPHYRIN LEVELS IN PATIENTS WITH VARIEGATE PORPHYRIA

Subject	Age	Attack or Remission	n	Urinary Sulphates		Urinary Glucuronides										Total Urinary	Plasma Sulphates				Total Plasma	Urine			
				E/A	A	E	DHA	EPI	A	E	DHA + EPI	11-OA	11-OE	11-OHA	11-OHE	17-Os	A	E	DHA	EPI	17-Os	ALA	PBG	Copro	Uro
Normal Controls	Range 40-49	Normal	10	0.87-2.31	0-0.37	0-0.53	0-0.75	0-0.39	0.11-1.41	0.28-1.78	0-0.25	0-0.21	0.04-0.85	0.03-1.45	0.01-0.45	0.6-6.7									
Case 40	53	R	2*		0.01	0.19	0.07	0.05	0.13	0.12	0.01	0	0.13	0.06	0.03	0.8	-	-	-	-	-				
Normal Controls	Range 20-29	Normal	15	0.19-1.02	0.24-3.74	0.05-0.94	0.20-5.69	0.14-0.76	2.04-10.85	0.69-7.19	0.02-4.44	0.10-0.53	0.18-1.55	0.67-2.16	0-1.26	7.0-26.0	28-114	0-21	58-356	6-40	98-502				
Case 41	24	R	1	0.70	1.31	0.23	1.98	0.22	3.13	2.87	0.17	0.30	0	1.65	0.48	13.3	95	0	174	20	287	A	A	A	A

n - No. of subjects in control group or no. of separate 24-hour urine collections from each patient
 * - Results expressed as the MEAN level of separate 24-hour urine collections
 The steroid levels in the urine and plasma were not elevated in either patient.
 A - Porphyrins or precursors elevated above the normal range

F. STEROIDAL STUDIES ON PATIENTS WITH NON-HEREDITARY
PORPHYRIA CUTANEA TARDA.

Non-hereditary porphyria cutanea tarda (PCT), also known as symptomatic porphyria, is a hepatic porphyria which is often associated with alcoholism. In this study, a total of fourteen patients (eleven males and three females) were investigated. The diagnosis of PCT was based on the typical clinical picture, skin sensitivity to 400 nm radiation, evidence of liver disease and a high urinary excretion of uroporphyrin and, to a lesser extent, coproporphyrin. Determination of the urinary excretion of the fractionated 17-OS conjugates revealed no abnormally high levels of any of these steroids in this entire group (Table 39). Similarly, determinations of the 17-OS sulphates in the plasma of these patients revealed no abnormally high concentrations of these steroids. The mean urinary E/A ratio for the eleven males with PCT was 0.77 ± 0.39 , which is not significantly different from that found for normal males. The mean E/A ratio for females with PCT was also found not to differ significantly from that for normal females. Thus, it seems that the steroid abnormalities observed in other hepatic porphyrias, AIP and HCP, are not to be found in PCT (Fig. 41). However, it must be noted that in 80% of the PCT patients examined, the total urinary excretion of the 17-OS conjugates was lower than the mean total urinary excretion of normals from that same age group,

TABLE 39

17-OXOSTEROID AND PORPHYRIN LEVELS IN PATIENTS WITH PORPHYRIA CUTANEA TARDA

				Urinary Ratio		Urinary Sulphates (mg/24 hr.)										Urinary Glucuronides (mg/24 hr.)										Total Urinary		Plasma Sulphates (µg/100 ml.)				Total Plasma		Urine			
Subject	Sex	Age	n	E/A	A	E	DHA	EPI	A	E	DHA+EPI	11-OA	11-OE	11-OHA	11-OHE	17-O5	A	E	DHA	EPI	17-O5	ALA	PBG	Copro	Uro												
Normal Controls	♂	Range 40-63	10	0.37-1.89	0.05-0.85	0.03-0.36	0.02-2.27	0.02-0.29	0.11-3.70	0-2.20	0.81-0.07	0-0.85	0-1.03	0.16-2.45	0-1.10	1.3-12.1	5-77	0-10	0-132	2-25	14-238																
Case 42	..	43	1	0.50	0.22	0.03	0.75	0.03	0.08	0.12	0.07	0	0.07	0.14	0	1.5	54	10	85	17	172			Λ	Λ												
Case 43	..	46	1	0.37	0.59	0.16	0.44	0.28	0.95	0.41	0.08	0.08	0.22	1.23	0.54	5.0	54	10	85	17	172			Λ	Λ												
Case 44	..	46	3*	1.54	0.05	0.11	0.06	0.12	0.45	0.65	0.05	0.13	0.33	0.34	0.18	2.5	16	0	33	8	57			Λ	Λ												
Case 45	..	48	3*	0.70	0.21	0.09	0.50	0.16	1.01	0.76	0.29	0.09	0.30	0.57	0.60	4.6	35	5	62	14	116			Λ	Λ												
Case 46	..	57	1	0.70	0.31	0.16	0.21	0.17	0.50	0.41	0.08	0.03	0.08	0.52	0.40	2.9	32	6	49	17	104			Λ	Λ												
Case 47	..	59	3*	1.41	0.09	0.17	0.17	0.14	1.07	1.45	0.07	0.18	0.88	1.23	0.91	6.3								Λ	Λ												
Case 48	..	50	1	0.98	0.12	0.03	0.02	0.23	0.21	0.20	0.04	0	0.24	0.59	0.13	1.8								Λ	Λ												
Case 49	..	51	1	0.87	0.24	0.17	0.09	0.10	1.41	1.26	0.04	0.16	0.61	0.85	0.28	5.2	57	4	102	25	188			Λ	Λ												
Case 50	..	63	1	0.23	0.48	0.12	0.31	0.16	0.94	0.20	0.06	0.06	0.16	0.62	0.32	3.4	34	0	70	28	132			Λ	Λ												
Case 51	..	66	1	0.80	0.09	0.12	0.07	0.03	0.30	0.18	0.04	0.07	0.21	0.20	0.30	1.6	24	2	58	10	94			Λ	Λ												
Case 52	..	67	1	0.47	0.13	0.03	0.36	0.10	0.82	0.41	0.02	0	0.14	0.50	0.36	2.9	25	6	55	19	105			Λ	Λ												
Normal Controls	♀	Range 40-49	10	0.87-2.31	0-0.37	0-0.53	0-0.75	0-0.39	0.11-1.41	0.28-1.78	0-0.25	0-0.21	0.04-0.85	0.03-1.45	0.01-0.45	0.6-6.7	5-42	0-8	16-85	0-19	35-127																
Case 53	..	47	4*	1.33	0.07	0.04	0.01	0.34	0.16	0.21	0.04	0.01	0.30	0.20	0.29	1.8								Λ	Λ												
Case 54	..	56	1	0.94	0.04	0.10	0.08	0.02	0.36	0.27	0	0.13	0.35	0.46	0.49	2.3								Λ	Λ												
Case 55	..	69	1	0.88	0.36	0.29	0.19	0.39	0.15	0.16	0.08	0.13	0.48	0.78	0.41	3.4								Λ	Λ												

n - No. of subjects in control group or no. of separate 24-hour urine collections from each patient.

* - These results are mean values of separate 24-hour collections.

All steroid levels within the normal range.

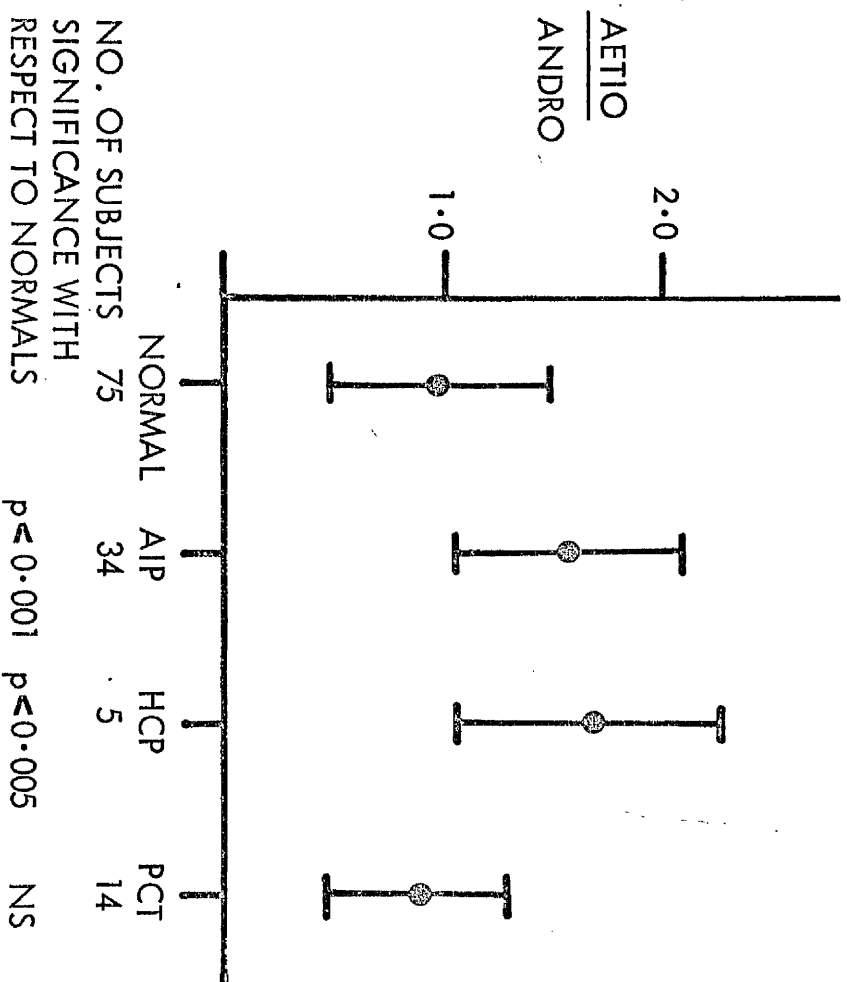
A - Porphyrins or precursors elevated above the normal range.

Figure 41

RATIOS OF URINARY AETIOCHOLANOLONE : ANDROSTERONE

IN HEPATIC PORPHYRIA

MEAN \pm S.D.



whereas in the plasma the reverse is true, with 89% of the PCT patients whose plasma was examined having a greater level of total 17-OS sulphates than the mean total level of sulphate conjugates in the plasma of normals from the same age group. A possible explanation for this observation is put forward in the discussion section of this thesis.

SECTION IX

DISCUSSION

DISCUSSION

The results of these studies imply that there is a close association between the hereditary hepatic porphyrias and abnormal steroid metabolism. The earlier report by Goldberg et al. (1969) that certain of the 17-OS conjugates in the urine of acute intermittent porphyrics are significantly elevated during attack, is extended to include patients suffering from hereditary coproporphyria. There are several theories which might explain these increased urinary excretions of specific 17-OS conjugates. They could be caused by an increase in the metabolic clearance rate due to a decrease in the degree of plasma protein binding of the steroids. It is possible to hypothesise that perhaps there are differences in the degree of protein binding of various steroids in normals and in patients with hereditary porphyria. However, the detection in this investigation of increases in the concentrations of some of the sulphate conjugates of these 17-OS in plasma suggests that the urinary elevations are not due to increased excretion rates but are caused by increased production of a steroid or steroids. Care has to be exercised in comparing the 17-OS sulphate levels in the plasma with the urinary excretion of the conjugates of the 17-OS, since these conjugates can have a number of origins other than the plasma 17-OS sulphates. Although the major components of total plasma 17-OS are AS and DHAS, the contribution to the total urinary 17-OS excretion of the minor quantities of free 17-OS can be considerable. This is due to the vast

differences in the metabolic clearance rates (Tait, 1963) between the free steroids and the steroid sulphates (Sandberg et al., 1964; Wang et al., 1967; Wang and Bulbrook, 1968). Wang and his colleagues (1967) reported that the ratios of the metabolic clearance rates of DHA and testosterone and their respective sulphates were approximately 40, indicating that the steroid sulphates are cleared much more slowly than the free compounds. Plager (1965) has reported that the sulphates of DHA, androsterone and aetiocholanolone are bound to human plasma albumin, which has a large number of binding sites which have a great affinity for steroid sulphates. These observations suggest the possibility that steroid sulphates in plasma act as physiological reservoirs for the unconjugated steroids. Thus, DHAS has been shown to be converted reversibly in vivo to DHA (Roberts et al., 1961; Sandberg et al., 1964; Slaunwhite et al., 1967), and more recently Rosenfeld and his colleagues (1972) have demonstrated that the major source of the plasma free DHA is production from DHAS.

The significance of the elevations of the concentrations of the plasma 17-OS sulphates is difficult to assess. It is generally agreed that the physiologically active steroid is the free unconjugated form and Plager (1965) has reported that 11 mg of DHAS are required to increase the concentration of protein-free (i.e. unbound) DHAS by 1%. Thus, the possibility arises that these plasma elevations detected in the porphyric patients are just increases in the reservoir of protein bound conjugated steroids, and thus have no physiological effect.

This criticism can be partially answered by the fact that aetiocholanolone glucuronide was one of the steroids whose urinary level was most consistently raised. Consistent elevations were also detected in the urinary levels of DHAG + EPIG. These observations imply that elevated levels of the free 17-OS do exist in the liver, since the liver is the site for steroid glucuronidation.

Another very important question which arises, is whether these steroid elevations are an effect or the cause of the acute attacks observed in the hereditary hepatic porphyrias. The fact that the levels of these 17-OS do in many cases remain elevated for many years after an acute attack, indicates that these elevations are not brought about by increased pituitary-adrenal activity due to the stress of an attack. Further evidence to support this view is provided by the observation that the 11-oxygenated-17-OS, which are mainly metabolised from the adrenal secreted 11 β -hydroxyandrostenedione and hydrocortisone which are raised in humans in stressful situations (Mason, 1968), are found to be normal in the majority of these patients in which the 11-deoxy-17-OS elevations were detected. These findings suggest that there is a long-term abnormality in steroid metabolism present in patients with hereditary hepatic porphyria, especially AIP. This does not rule out the possibility that stress does play some part in the provocation of the acute attack in susceptible individuals. Elevations in the urinary excretion of 17-OS have been reported to occur in humans involved in such stressful situations as

air-craft flight (Pincus and Hoagland, 1943), emergency hospital duty (Pincus, 1947), motor-racing (Frost et al., 1951), final examinations (Connel et al., 1958). Similar, and in some cases more pronounced elevations in the levels of the 17-hydroxycorticosteroids in urine and in plasma have been detected in a variety of psychological and emotional disturbances by a large number of investigators (for review, see Mason, 1968). It is possible in those patients in whom no exogenous factors such as drugs, contraceptive steroids, alcohol or a diet very low in carbohydrate and protein, may be implicated with the onset of an acute attack, some stressful situation or emotional disturbance could result in stimulation of ACTH secretion leading to increased production of 17-OS and hydrocortisone. The already high concentrations of circulating 17-OS sulphates in the plasma could be intensified to such an extent that onset of an acute attack would result. Birke et al. (1955) have reported that post-operative values of the urinary excretion of DHAS are greatly elevated compared to normal, and it might be worthwhile to note that in many patients with hereditary hepatic porphyria, the first acute attack occurred after some surgical procedure (Goldberg et al., 1969; Eales, 1971; Case 39, this Thesis). However, in the majority of these cases, the patient had undergone thiopentone anaesthesia or had received drug therapy in the post-operative phase, and it seems more likely that the main precipitating factor in the acute attack was the drug, especially if it was a barbiturate. This does not rule out the possibility that

increase of adrenal activity due to operative trauma was a contributory factor in provocation or aggravation of the acute attack. In his study of 145 attacks in 120 patients suffering from VP and AIP, Eales (1971) found fifteen examples of attacks being precipitated or aggravated by emotional stress in four individuals. In this publication, Eales also reported that many of these patients who had suffered acute attacks after thiopentone anaesthesia had undergone previous thiopentone anaesthesia, often repeatedly, without ill effect. These observations indicate that, although the cardinal role of the barbiturates in the genesis of the severe attacks has been established, there are nevertheless other unknown contributory factors, possibly hormonal in nature, which must be accounted for.

The concept of permissibility of hormone action has been extensively reviewed by Ingle (1954). The permissive effect of the glucocorticoid hydrocortisone on the induction of ALA synthetase by AIA in the liver of adrenalectomized animals has been demonstrated by Marver et al. (1966c). They found that adrenalectomized mice did not become porphyric when treated with AIA, but only when treated with both AIA and hydrocortisone. Although the evidence indicates that the glucocorticoids cannot themselves induce, they may be important in bringing about the metabolic conditions to permit the inducing steroids or chemicals to induce. Matsuoko et al. (1968) suggested that hydrocortisone potentiated the action of drugs, since in normal intact rats the steroid increased the extent of induction by sub-optimal doses

of AIA and, if given one hour before AIA, it reduced the lag period preceding the increase in ALA.S activity. These observations, together with the facts that adrenalectomy prevents AIA-induced proliferation of the smooth endoplasmic reticulum (Moses et al., 1970) and AIA-induction of hepatic tyrosine and tryptophan transaminases (Wetterberg et al., 1970) are consistent with a permissive role for hydrocortisone which is known to have general stimulatory effects on hepatic RNA and protein synthesis as well as causing relative specific induction of some enzymes (see Cox and Mathias, 1969, for review). Of interest concerning the action of hydrocortisone in this connection is the study of Mills and Topper (1969) who found that 4-day old explants of mammary alveolar epithelial cells require hydrocortisone for the formation of an extensive rough endoplasmic reticulum which is needed for the synthesis of casein. Also of interest, are the electron-microscopic observations made by Rancourt and Litwack (1968), whose results indicated that hydrocortisone, as early as 15 minutes after injection, provoked changes in the endoplasmic reticulum involving conversion of much of the long parallel arrays of rough endoplasmic reticulum to vesiculated smooth endoplasmic reticulum. These experiments suggest that the glucocorticoids may be active in the formation of changes in the endoplasmic reticulum, and these changes may have to precede the induction of some of the inducible liver enzymes. In the chick embryo liver tissue culture system, there is apparently no requirement for hydrocortisone; neither does hydrocortisone cause induction

of porphyrin synthesis (Granick, 1966). Although adrenalectomy prevented induction of ALA.S and the drug detoxifying system by AIA in rat liver, it did not prevent induction of the drug metabolising enzymes by phenobarbitone (Ichii and Yago, 1969; Marshall and MacLean, 1969; Marshall, 1971). These phenobarbitone-stimulated increases in cytochrome P450, cytochrome b₅, microsomal protein, phospholipid and NADPH-cytochromic reductases were completely blocked by Haemin (Marver, 1969).

It is possible to hypothesize several relationships between these glucocorticoids and the effects of some of these porphyrinogenic drugs. These glucocorticoids may act by enhancing the activity of the inducer or by bringing about metabolic conditions which permit the inducer to induce. It is known that the production of haem may be enhanced by agents which stimulate gluconeogenesis (Rose et al., 1961). The stress of fasting may increase the synthesis and secretion of glucocorticoids, which increases the activity of a number of liver enzymes concerned with gluconeogenesis and, which permit more readily induction of ALA synthetase. The studies of Drews and Brawerman (1967) suggest that this involvement of the glucocorticoids in the induction of a number of liver enzymes is due to their effect on the mRNA's. Using DNA-RNA hybridization, they observed that 3 hours after hydrocortisone administration to adult rats, the mRNA's of the liver were altered, implicating an early selective effect of the hormone on the synthesis of mRNA's coding for the relatively few enzymes that were induced. A different interpretation of

glucocorticoid action on the induction of certain liver enzymes has been suggested by Tomkins et al., (1969). They proposed that the steroid acts not at the transcriptional level but rather at the translational level to inhibit the action of a repressor on the synthesis of the enzyme.

It also must be noted that drugs and steroids are metabolised by the same cytochrome P-450-containing enzyme system in the liver microsomes and thus competition for the binding sites of P-450 would result (Conney et al., 1968; Tephly and Mannering, 1968; Kupfer and Orrenius, 1970). By competitive inhibition of steroid hydroxylation, drugs like AIA, DDC and phenobarbital, which are strong porphyrinogenic drugs and have been shown to bind strongly to P-450 (De Matteis, 1970; Tschudy and Bonkowsky, 1972), might enhance metabolism of steroids by pathways leading to production and accumulation of active inducers. However, the numerous studies of the effects of AIA and DDC on hepatic enzymes and metabolic pathways other than those of porphyrin biosynthesis, provide evidence for far-reaching and numerous metabolic effects of these substances (see Tschudy and Bonkowsky, 1972, for review). This myriad of changes in the levels of enzymes brought about by porphyrinogenic drugs, as well as the consideration of the changes in steroid metabolism, raises the fundamental question as to which changes are related to porphyria and which are unrelated?

One way of attempting to answer this question is to determine which of the biochemical changes observed in

experimental porphyria also occur in the most closely analogous type of genetically determined hepatic porphyria. Unfortunately, there is a lack of study of steroid metabolism in experimental porphyria, but an example of such a correlation is observed in the abnormalities of lipid metabolism which occur in both the experimental disease and variegate porphyria (Taddeini et al., 1964). In the experimental porphyria, the following observations have been made: rats treated with AIA showed an increase of hepatic fatty acid synthesis (Labbe et al., 1961); rabbits treated with AIA or DDC develop increased serum levels of total lipids and phospholipids (Taddeini et al., 1964); mice treated with AIA or DDC or griseofulvin develop hypercholesterolaemia due to increased hepatic synthesis (De Matteis, 1966; Wada et al., 1969). Hypercholesterolaemia is also found in VP and in approximately 40% of patients with AIP (Taddeini et al., 1964; Stein and Tschudy, 1970). It is of interest to note that hypercholesterolaemia and hyperlipaemia in patients have for some time now been associated with abnormal steroid metabolism. Hellman et al. (1959) were amongst the first to suggest a role of androgen hormones in cholesterol metabolism. A significant increase in epiandrosterone excretion by hypercholesterolaemic subjects has been reported by Dingman and Lim (1963), who suggested that the abnormality was due either to a derangement in the steroid hydroxylating enzymes responsible for the hepatic degradation of androgenic hormones or to an increased secretion of a specific androgen. In contrast to this, Adlercreutz et al. (1968; 1972) observed a tendency towards lower plasma levels and urinary excretion of several

steroids, especially DHAS in a small group of patients with hypercholesterolaemia or hyperlipaemia. These investigators stated the need for further investigation to clarify whether the alterations in steroid metabolism in the hypercholesterolaemic and hyperlipaemic subjects were of aetiological significance or were only a secondary or parallel phenomena to the disturbances of lipid metabolism or vascular changes common in these diseases. An exactly similar problem exists in the porphyrias in assessing whether the alterations in steroid metabolism are of aetiological significance or not. A number of clinical observations do suggest that endocrine secretions, particularly steroids, represent one class of natural agents which in appropriate circumstances may exacerbate the chemical and symptomatic abnormalities of hepatic porphyria. These observations are: the onset of the acute attack seldom occurs before the age of puberty; there is a female predominance; many of the acute attacks in women are associated with the luteal phase of the menstrual cycle or with pregnancy; the main age of onset is in the third decade for females and the third or fourth for males; the disease rarely occurs after middle age; chemical as well as symptomatic relapse results as a consequence of sex hormone administration in some patients. It was these striking clinical associations which led Granick and Kappas (1967a,b; 1968) to examine the possibility that naturally occurring steroids might be involved in the regulation of ALA synthetase, the rate controlling enzyme of haem synthesis. In normal liver, this enzyme is closely controlled by some fine mechanism as the

hepatic haem requirement in the liver is fairly small, being about 15% of that required by the bone marrow to replace the haemoglobin lost through red cell breakdown. In adult bone marrow, approximately 210 mg of haem are formed daily. For this amount of haem, the ALA.S has to make 358 mg of ALA. In the hereditary hepatic porphyrias, the liver may readily produce this much and more ALA (Granick and Sassa, 1971). These investigators postulated that, when the requirement for haem was increased by the drug detoxifying system, the hepatic ALA synthetase activity could be stimulated by certain endogenous steroids, resulting in increased synthesis of ALA, culminating in an increase in haem. They even suggested that there may be some natural steroids that normally serve to maintain a certain rate of haem synthesis by inducing the synthesis of ALA synthetase.

During the past few years, evidence has accumulated showing that a number of natural steroid metabolites of the 5β -H type, such as aetiocholanolone, aetiocholandiol, pregnandiol, pregnantriol and pregnanolone, induce the de novo synthesis of ALA synthetase in chick embryonic liver cell in vivo and in vitro, and thus stimulate porphyrin synthesis. Similar results with 5β -H steroids have been obtained recently in mammalian systems, including bone marrow cells from human subjects and from mice and rats (Necheles and Rai, 1969; Gordon et al., 1970; Sardesai et al., 1970). The porphyrin-inducing action of some of these steroids was maximal at concentrations as low as 10^{-5} M, and hence was as great as the most active of the non-steroid porphyrinogenic compounds

previously studied. Furthermore, in these studies Granick and Kappas showed that the glucuronide derivatives of even potent inducers are devoid of activity and that steroid induction of ALA is inhibited by concomitant addition of haem and certain other metalloporphyrins. The significance of these steroidal studies is very great, as they demonstrate that a number of steroids which were previously considered to be waste metabolites of adrenocortical and gonadal hormones with no primary function of their own may, in fact, exert considerable influence over haem synthesis in liver and erythroid cells, and it was on the basis of these studies that Granick and Kappas (1967a) applied the operon concept of Jacob and Monod (1961) to an hypothesis explaining the regulation of haem biosynthesis by steroids in the hepatic cells. In this hypothesis, they suggest that any process which led to (a) impaired glucuronidation of the steroids, such as inhibited or defective UDP-glucuronyl transferase or limited UDPGA formation, or (b) enhanced intracellular hydrolysis of formed conjugates by excessive β -glucuronidase activity, or (c) steroid production in amounts exceeding the body's capacity to dispose of them, would lead to induction of ALA synthetase and increased production of ALA and other precursors of haem. This original hypothesis was further modified into the form shown in Figure 17, when further investigations indicated that haem represses the synthesis of ALA.S and thus its own synthesis at the translational level rather than at the transcriptional level. However, the recent finding of Schneck et al. (1971) that haem had no apparent effect

on porphyrin synthesis when added to chick embryo liver cultures preinduced with AIA has raised some doubt that this hypothesis is always valid. Similarly, in rat liver it has been reported by Kurashima et al. (1970) that haem administered 2 - 10 hours after AIA does not decrease total induction by AIA but alters only the relative proportions of enzyme in mitochondrial and cytoplasmic fractions of the cell by preventing appearance of newly synthesised enzyme in the mitochondria. A redistribution phenomenon of this type may have been the post-transcriptional effect of haem observed by Sassa and Granick (1970), who apparently measured ALA.S activity in isolated mitochondria. It may be of interest at this point to note that steroids have been reported to affect the permeability of mitochondria (Gallagher, 1960).

After Granick and Urata's discovery in 1963 that hepatic ALA.S is an inducible enzyme, the possibility that a genetically mediated induction of this enzyme might explain the excessive excretion of porphyrin precursors in AIP was investigated (Tschudy et al., 1965; Nakoa et al., 1966; Perlroth et al., 1966; Dowdle et al., 1967). Their findings demonstrated that a mutation in man can augment the expression of a specific enzyme. However, it is still not known whether the mutation is primarily in a control gene or whether the induction of hepatic ALA.S is secondary to a structural gene mutation. Tschudy et al. (1965) postulated that the genetic abnormality in AIP was a defect of a regulator gene which normally controls the synthesis of ALA.S. A similar lack of repression of the

synthesis of ALA.S related to a constitutive regulator operon mutation, in accord with the concept of Jacob and Monod, is suggested by Watson et al. (1964b) to provide a reasonable explanation of the genetic and biochemical manifestations of erythropoietic porphyria. The resemblance of the clinical and biochemical features of AIP to those of VP and HCP suggests that a similar metabolic abnormality must be present in all three, and it seems doubtful whether a single regulator-gene defect could account for the differences in the pattern of porphyrin excretion which is observed in the three conditions (see Fig. 8). Tschudy and Bonkowsky (1972) have suggested that, since induction of ALA.S in liver by compounds which cause experimental porphyria may result in 50-fold or greater increase in the level of this enzyme, under these conditions the other enzymes beyond ALA.S in the pathway which are normally present in excess become rate limiting for haem biosynthesis, and the patterns of metabolites accumulated and excreted may reflect the relative levels of these enzymes. This hypothesis appears unlikely to explain more than one form of genetically transmitted porphyria since, with rare exceptions (Taddeini and Watson, 1968), excretory patterns are reported to be 'true to form' in families with each of these disorders (Waldenström, 1957; Schmid, 1966). Thus, if a mutation leading to increased ALA synthesis were to explain all these disorders, this defect would have to be combined with additional mutations (which is statistically improbable) to explain the different excretory patterns of porphyrins and precursors which typify each disorder.

An alternative to this hypothesis was proposed by Kaufman and Marver (1970), who suggested that each of the porphyrias primarily results from partial blockage in haem biosynthesis at a locus distal to ALA. In view of the role of haem in the feed-back regulation of ALA.S (Granick, 1966; Kappas and Granick, 1968; Marver et al., 1968; Hayashi et al., 1968; Sassa and Granick, 1970), the partial block in haem biosynthesis may result in a decrease in the level of available haem, resulting in secondary induction of ALA.S. The marked increase in the porphyrin precursors, ALA and PBG, in relation to the normal or near normal porphyrins in the urines of patients with AIP, suggests that the conversion of PBG to uroporphyrinogen by the enzyme URO-I-synthetase is defective. Heilmeyer and Clotten (1969) observed a decreased hepatic production of porphyrins from ALA in patients with AIP, but Nakao et al. (1966) reported that PBG disappearance catalysed by liver homogenate from a patient with AIP was similar to that of controls. In the case of HCP, the defect would presumably be in the conversion of coproporphyrinogen III to protoporphyrinogen by the enzyme coproporphyrinogen oxidase, and in VP the conversion of protoporphyrinogen to haem. These partial blocks of haem biosynthesis could be the consequence of diminished activity of enzymes involved at specific steps in the pathway, loss of intermediates due to alterations in membrane permeability of the hepatocytes (Taddeini and Watson, 1968) or the mitochondria, or the excessive irreversible conversion of the reduced porphyrinogens to the oxidised porphyrins. It was Falk et al. (1959) who

first demonstrated that oxygen tension influences different biosynthetic steps in different ways, and thus the Ox/Redox environment within subcellular organelles may play a regulatory role. Thus, the transformation of coproporphyrinogen III to protoporphyrinogen is oxygen dependent, whilst chelation of protoporphyrin with iron to form haem is favoured by relatively anaerobic conditions. Rimington (1963) has also postulated that the increased excretion of porphyrins in PCT result from excessive oxidation of porphyrinogens and diversion of these intermediates from haem biosynthesis. This same author (1966) has also pointed out the fact that some of the enzymic steps of the haem biosynthetic pathway take place within the mitochondria and others in the cytoplasm, and thus intermediates have per force to diffuse from one location to another. This alone can act as a rate limiting and regulating mechanism, especially as substrates may at some stages be protein bound and their diffusion will thus be modified accordingly, perhaps even resulting in a partial block of the haem biosynthetic pathway.

This hypothesis of partial block in haem biosynthesis proposed by Kaufman and Marver (1970) has been argued against on the basis that haem synthesis is actually increased in experimental porphyria (Marver, 1969). However, this experimental model need not be completely relevant to the human genetic disease. Recent investigations by Strand et al. (1970) have confirmed that AIP is indeed distinguished from VP and HCP by decreased activity of hepatic URO-I-synthetase.

This does not rule out the possibility, however, that in families with AIP decreased URO-I-synthetase constitutes an unrelated variation in enzyme activity that may modulate primary over-production of ALA. Such genetically determined relatively common variations in enzyme proteins occur in heterogeneous populations and are referred to as enzyme polymorphisms (Harris, 1970). Further investigation in a large family with AIP by Meyer et al. (1972), provided further data which suggested that decreased URO-I-synthetase activity reflected the primary genetic defect in AIP, which then may result in secondary induction of hepatic ALA synthetase, leading to over-production of ALA and PBG. This hypothesis of a partial block in haem biosynthesis also at the moment seems to provide an acceptable explanation for the primary abnormality in the other two forms of hereditary hepatic porphyria, with the secondary elevation of ALA.S accounting for the increase in ALA and PBG excretion which occurs in both during attack and the site of the block explaining the differing pattern of porphyrin excretion.

The acceptance of this hypothesis raises the question concerning the role of the steroids in the aetiology of the porphyrias; namely, does the involvement of steroids require the postulation of another metabolic abnormality in the hepatic porphyrias or is it possible to explain the steroidal abnormalities observed within the frame-work of Kaufman and Marver's hypothesis? Does the enzymic change observed by Gillette et al. (1970) in AIP patients result from a genetic change and, if so, are there two enzyme defects, one basic and one accessory, involved in some cases of AIP?

In 1970, Gillette et al. reported that in AIP patients the 5α -H pathway for steroid metabolism was greatly diminished, indicating a gross deficiency of steroid Δ^4 - 5α -reductase activity, which resulted in preferential formation of the 5β -H type metabolites. This finding would seem to agree with the results of this thesis obtained in the studies on the urinary levels of the 17-OS conjugates. In these studies, the ratio of the urinary level of aetiocholanolone (5β -H steroid) to the urinary level of androsterone (5α -H steroid) was found to be significantly greater for the patients who were suffering from hereditary hepatic porphyria (AIP and HCP investigated) than for the normals or for patients with acquired porphyria cutanea tarda. During an acute attack, the E/A ratio was observed usually to be at a maximum. These results suggest that hereditary hepatic porphyrics have an imbalance in their steroid metabolism, resulting in an overall increase in the 5β -H type steroid metabolite. However, this specific abnormality of steroid metabolism in porphyrics is probably not due to direct genetic change affecting the activity of the Δ^4 - 5α -reductase but more likely to be caused by the indirect effects of various precursors and hormonal influences to which the Δ^4 -reductases are very susceptible. As a result of these influences, which include thyroid hormone (Hellman et al., 1959; McGuire and Thomkins, 1959), glucocorticoids (Wilson and Schenker, 1964), the urinary E/A ratio may be shifted. One such endogenous compound which might greatly influence the E/A ratio, especially in the hereditary hepatic porphyrias, is DHA which has been

reported to suppress Δ^4 -5 α -reductase (Kirschner and Lipsett, 1964) and which is one of the main plasma precursors of the urinary androsterone and aetiocholanolone (Baulieu and Mauvais-Jarvis, 1964a,b). The sulphate conjugate of DHA was also the 17-OS in the plasma which was the most frequently and most strikingly raised in the steroid investigations in the plasma of the hereditary hepatic porphyric patients. This increase in the plasma levels of DHAS, resulting in an increase in the level of free DHA in the liver, leading to suppression of Δ^4 -5 α -reductase, provides a possible explanation for the deficiency of Δ^4 -5 α -reductase observed by Gillette et al. (1970) and Kappas et al. (1971) in AIP patients and the significant increases in the urinary E/A ratios of the hereditary hepatic porphyrics examined in this thesis. It may be of interest to note that DHA has been reported to inhibit glucose-6-phosphate dehydrogenase activity, with a resultant decrease in the synthesis of NADPH which is required by the Δ^4 -reductases (Lopez and Krehl, 1967). It is possible that this mechanism plays a part in the inhibition of the Δ^4 -5 α -reductase activity or perhaps helps in the accumulation of steroids in the liver. Although these 5 β -H type steroids appear to be produced in excess due to a secondary effect of some earlier abnormality in the steroid pathway, i.e. overproduction of DHA or DHAS, there is the possibility that they do have an aggravating role to play in hepatic haem synthesis. A number of groups have shown that the 5 β -H type steroids, known to be potent

inducers of ALA synthetase in chick embryo liver and erythropoietic tissue (Granick and Kappas, 1967a,b; Levere et al., 1967), also stimulate haem and globin synthesis in mammalian bone marrow (Necheles and Rai, 1969; Sardesai et al., 1970; Levere and Mizoguchi, 1971). However, attempts to demonstrate induction by these steroids in mammalian liver have been unsuccessful (Song et al., 1970; Sardesai et al., 1970; Tschudy and Bonkowsky, 1972), perhaps because of some difficulty in getting the relatively polar steroids to accumulate in the liver cells in adequate concentrations. There is also the possibility that in mammalian liver a more specific structure or even a completely different structure is required for the inducing steroid. In their studies of perfused rat liver, Bock and his colleagues (1971) found that the sharp decrease observed in the activity of ALA synthetase could not be prevented by testosterone or aetiocholanolone, whereas the C-21-hydroxylated steroids, dexamethasone and hydrocortisone, were able to maintain the synthetase at the level, in vivo. Despite these findings in the rat liver, the results of this thesis suggest that the 5β -H type steroids or their precursors do play some part in the induction of the increased activity of ALA synthetase observed in AIP and HCP. In the two cases of HCP which were examined in attack, fairly good linear correlation was obtained between the urinary E/A ratio and the urinary levels of ALA and PBG. The urinary E/A ratio was at its maximum during the attack when the levels of ALA and PBG in the urine were at their highest, and then gradually decreased with corresponding decreases in the levels of ALA

and PBG. Unlike AIP patients, the urinary levels of ALA and PBG in HCP are only elevated during an attack, whereas AIP patients may be in remission for many years and still be excreting excessive quantities of ALA and PBG in the urine. Of all these patients who were excreting excessive amounts of ALA and PBG, only one was found which did not show any elevations in any of the 17-OS measured, either in the urine or in the plasma. A similar linear correlation between the urinary E/A ratio and the excretion of ALA and PBG was found for one patient, Case 5, with AIP (Figure 35). As with the HCP patients, these correlations were found in urines collected during and after the acute attack. These observations do indicate that increased amounts of 5 β -H type 17-OS may play a part in bringing about the abnormal synthesis of porphyrins and precursors which occurs in the porphyrias. Unfortunately, the mechanism through which the effects of these steroids are mediated is completely unknown. Consideration of previous research in this field of investigation suggests that out of the many possible mechanisms, the two most likely are: the excess steroids would act directly as derepressors of the operon allowing de novo synthesis of ALA synthetase, or indirectly by inhibition at some later point in the haem biosynthetic pathway; perhaps bringing into effect the partial block in the pathway, as hypothesised by Kaufman and Marver (1970).

The observations made on Case 26, a male AIP, are suggestive that the steroid effect takes place by the former

mechanism. During attack, this patient excretes excessive amounts of ALA, PBG, uroporphyrin and coproporphyrin and excessive amounts of 17-OS conjugates, of which DHAS and EG were the most strikingly elevated. The fact that the amount of preformed uroporphyrin and coproporphyrin in the urine is significantly increased must be considered in respect to a partial block in the transformation of PBG to uroporphyrinogen by the enzyme URO-I-synthetase. The amount of uroporphyrin and coproporphyrin represents but a small percentage of the PBG formed. Thus, it is likely that the amount of activity in the liver, although significantly reduced, nevertheless suffices to convert a small proportion of the greatly increased PBG to uroporphyrinogen and thence to coproporphyrinogen. Since DHA and its sulphate have been shown to be quantitatively one of the main steroids secreted by the adrenal cortex (Baulieu et al., 1965) and since in the investigation of this patient the most striking steroid abnormality was the raised levels of DHA conjugates, it was suggested that treatment with dexamethasone, which suppresses ACTH release leading to reduction in the activity of the adrenal cortex (Sirett and Gibbs, 1969), might alleviate the symptoms of porphyria. After dexamethasone administration, there was a significant fall in the urinary levels of the 17-OS conjugates with a corresponding sharp and significant fall in the levels of both porphyrins and precursors. If DHA or one of its metabolites had been acting as an inhibitor at the point in the pathway of the conversion of PBG to uroporphyrin (i.e. the step at which Kaufman and Marver have suggested there is a partial

block of haem synthesis) resulting in secondary induction of ALA synthetase and increased production of ALA and PBG, a decrease in the level of this steroid inhibitor due to the dexamethasone effect would not have resulted in such a dramatic fall in uroporphyrin excretion, as was observed with this patient (see Table 36). Therefore, it seems likely that this steroid or one of its metabolites acts directly on the activity of the ALA synthetase. This decrease in the urinary excretion of the 17-OS conjugates and porphyrins and precursors was accompanied by a corresponding clinical improvement in the condition of the patient. The possibility that this lowering of porphyrin and precursor excretion and clinical improvement was a spontaneous event, independent of the administration of dexamethasone, must not be dismissed. There also exists the other possibility that its effect may be due to other factors in this system unconnected with steroids, since dexamethasone administration has been shown to lead to a significant depression of citric acid levels in humans (Wajchenberg et al., 1969). This depression of citrate could lead to a depression of succinate with a subsequent decrease in the entry of succinyl CoA substrate into the haem biosynthetic pathway.

This steroid, DHA and its sulphate, have been shown by Goldberg et al. (1969) to cause a striking rise of hepatic ALA synthetase when injected into rats. Further indirect evidence that some steroids or their metabolites are implicated in the control of ALA synthetase and porphyrin metabolism in

humans is provided by the reports of Koskelo et al. (1966) and Burton et al. (1967), which indicate that the oral contraceptive pill can upset porphyrin metabolism resulting in increased urinary excretion of porphyrins and precursors. Likewise, there are several reports of attacks of hereditary hepatic porphyria being provoked by oral contraceptives (Redeker, 1963; Wetterberg, 1964; Dean, 1965; Baxter and Permowicz, 1967; McKenzie and Acharya, 1972). Kappas et al. (1969) have also shown that plasma taken from women on the contraceptive pill and from AIP patients in attack or remission caused an induction of porphyrins which was blocked by UDP glucuronic acid addition in chick tissue culture. Plasma taken from normal individuals had no effect on porphyrin production in the tissue culture. Despite these suggestive findings, it remains to be shown whether sex steroids or some endogenous steroid metabolite directly cause the induction of ALA synthetase in human liver. Most of the steroidal observations in vivo could conceivably be explained, for instance, by inhibition by these steroids at some later point in the haem biosynthetic pathway with secondary induction and overproduction resulting from this, but the observations on the effects of dexamethasone on one male AIP patient in this thesis and the overall concept of the mechanism of the steroid effect resulting from the in vitro investigations of a large number of different groups, do suggest that the effect of the steroid on hepatic haem synthesis in human porphyria is mediated through an induction process with the specific steroid acting as a derepressor of the operon, resulting in de novo

synthesis of the enzyme ALA synthetase, with the genetically determined partial blocks in the haem synthesis in the liver explaining the different porphyrin and precursor excretion patterns which are characteristic of each of the diseases, as hypothesised by Kaufman and Marver (1970).

In their examination of a large family with AIP, Meyer et al. (1972) discovered two seemingly unaffected siblings with normal urinary excretion of ALA and PBG but who had low erythrocyte URO-I-synthetase similar to their affected siblings. In one of these two siblings, an acute attack of AIP developed spontaneously six months after completion of their study. Similarly, no clinical or chemical manifestations of porphyria could be found in the parents, but the father was identified as the transmitter of the genetic defect by the finding that his erythrocyte URO-I-synthetase activity was reduced to a level comparable to that of the siblings with manifest porphyria. These examples are good illustrations of the wide variability in the expression of this genetic disorder, and points to the probable role of some other factor in bringing out the clinical and chemical manifestations of the disease. In all possibility, this other factor could be the involvement of the adrenal cortex (or even the hypothalamus) and steroid metabolism with the porphyrias. A wide variety of different factors, including a large number of different drugs (barbiturates, sulphonamides, griseofulvin, dichlorophenazone, oral contraceptives), alcohol abuse, infections, dietary factors, hormonal factors, pregnancy, sunburn and emotional stress, have all been implicated as

precipitating and aggravating factors in acute episodes of the hepatic porphyrias (Eales, 1971), and there is the distinct and attractive possibility that their effects are mediated through interaction with an endogenous regulatory system of steroid metabolism in the liver. The following factors are known to act directly on steroid metabolism in the liver: sexual state, stage of development, treatment with hormones (sex hormones, anabolic steroids, corticosteroids, thyroid hormone), a wide variety of drugs, state of nourishment, conditions of stress and the rate of blood flow through the liver (for review, see Schriefers, 1969). This list is very similar to that of factors involved in the precipitation of hepatic porphyria, and it is possible that these forementioned factors result in the over-production or accumulation of a specific steroid metabolite with strong ALA.S inducing activity. In normals, this would result in an increase in production of haem, leading to an increase of the haemoproteins (P-450), which are components of the microsomal drug and steroid detoxification system, ultimately resulting in increased polarisation of the specific steroid metabolite, conjugation and excretion. In patients with porphyria who have inherited a partial block in haem synthesis, this induction effect of the steroid would just result in accumulation and excretion of the precursors formed previous to the partial block with, perhaps, a slight increase in the production of haem, but not enough to produce the additional haem required for increasing the levels of these drug and steroid metabolising microsomal cytochromes for the

detoxification of the inducing steroid. This concept is supported by the fact that, when haem synthesis is partially inhibited by 3-amino-1,2,4-triazole (Tschudy and Collins, 1957), induction of P-450 by phenobarbital is impaired (Baron and Tephly, 1969). Beattie and Stuchell (1970) reported an increase in the cytochrome content of mammalian liver mitochondria after administration of AIA (a very potent porphyrinogenic compound in experimental animals), but this is not in agreement with the results of other investigators who have shown that AIA causes an increased turnover rate of microsomal haem in the liver (De Matteis, 1970; Meyer and Marver, 1971). Thus, AIA appears to prevent a rise of P-450 by augmenting turnover of either the total enzyme or its haem moiety. This failure to increase the level of drug and steroid metabolising enzymes allows an inducer to persist in the liver for a longer length of time. A similar situation could occur in hereditary hepatic porphyrics, with the failure to increase the level of drug-(steroid)-metabolising enzymes being due to the partial block in the pathway, and thus allowing the inducing steroid to persist in the liver for a longer length of time. If microsomal haem is involved in the repression of ALA synthetase, the above situation in porphyrics may result in a decreased haem pool which might interfere in the closed negative feedback loop by haem, which is assumed to operate in the repression of ALA.S, thus augmenting the original inducing effect of the steroid.

This type of steroid effect need not only apply for

hereditary porphyria but might also have an effect on acquired porphyrias, like PCT symptomata or the cutaneous porphyria which occurred after inadvertent ingestion of wheat containing the fungicide hexachlorobenzene, in many people in Turkey. Although the examination of the levels of the 17-OS in the urine and plasma of PCT patients in this thesis did not reveal any abnormally elevated levels, as in the hereditary hepatic porphyrias, there have been many reports by other investigators associating the administration of steroids, especially oestrogens, with PCT (Hurley and English, 1963; Warin, 1963; Becker, 1965; Copeman et al., 1966; Felsher and Redeker, 1966; Levere, 1966; Thivolet et al., 1967; Vail, 1967). The majority of these patients were given the hormonal preparation for prostate carcinoma, and an interval of several months or years had elapsed between the commencement of therapy and the appearance of cutaneous symptoms. Raised levels of hepatic ALA.S in PCT patients have been reported by Levere (1966) and by Moore et al. (1972b). It seems unlikely, due to the time interval between commencement of oestrogen administration and appearance of symptoms, that the oestrogens themselves induce the ALA.S. Granick and Kappas (1967b) reported that oestradiol and related naturally occurring and synthetic oestrogens are only weak inducers in chick liver cell culture. Later, in 1970, Rifkind et al. showed that the progestational components of the contraceptive pill induced hepatic ALA.S activity, and the inducing effect was not significantly altered by the addition of oestrogens. Although there is the possibility

that, due to species differences, oestradiol or related compounds might in humans have a potent porphyrinogenic inducing activity, it seems more probable that they exert more indirect effects; for example, on the levels of other inducers (steroid ?). Oestrogens are known, at least in rats, to decrease metabolism of some steroids and drugs in the microsomal drug metabolising system (Conney et al., 1965; Kato and Onoda, 1970) and to alter metabolism of steroids by other pathways (Yates et al., 1958; Chatterton et al., 1970), perhaps resulting in an increase in an inducing steroid. It is these indirect effects of the oestrogens which are probably responsible for the precipitation of mild attacks observed in AIP after administration of a variety of exogenous oestrogens (Redeker, 1963).

There is also evidence that prolonged administration of these oestrogenic compounds may impair liver excretory function (Combes et al., 1963; Mueller and Kappas, 1964; Kottra and Kappas, 1967). Eisalo et al. (1964) described impaired liver function after administration of oral contraceptives, apparently due to their oestrogenic component. A significant elevation of urinary coproporphyrin and ALA has also been found in pregnancy, especially when associated with hepatic disease (Koskelo and Toivonen, 1968) and in women taking oral contraceptives (Koskelo et al., 1966). These findings suggest that impairment of liver excretory function may be partly responsible for increased urinary excretion of porphyrins and precursors, and for the appearance of symptoms of photosensitivity in PCT.

This also raised the question of whether or not the mechanism of induction of an attack in hereditary porphyria by oestrogens may occur, in part at least, indirectly by establishing a condition of liver damage resulting in a lowering of conjugation and excretion of steroids. An indirect mechanism of this type may, perhaps, explain the reason for the length of time taken by the majority of these oestrogenic agents to cause increased porphyrin excretion and symptoms of PCT, and the reason for the persistence of the biochemical manifestations in spite of the withdrawal of the causative agent once the metabolic disorder is established (Becker, 1965; Copeman et al., 1966; Zimmerman et al., 1966). Further support for this suggestion is provided by the observations that the clinical and biochemical evidence of liver damage is much more common in PCT than in the other varieties of hepatic porphyria, and by the reports that increased porphyrin excretion and appearance of symptoms of photosensitivity are observed in chronic liver disorders in which there is no history of alcoholism, or of prolonged exposure to any of the hormonal preparations, drugs or toxic factors associated with hepatic porphyria (Brunsting, 1954; Tio et al., 1957; Braun and Berman, 1959).

Another important factor which may play a secondary aggravating role in PCT, is the faulty regulation of steroid conjugation resulting in an accumulation of free steroids in the liver. Shanley et al. (1969) have reported that conjugation of aetiocholanolone was found to be impaired both in patients with symptomatic porphyria (PCT) and in non-porphyric patients with liver disease, compared to normal subjects.

Similarly, Zumoff et al. (1966; 1967) found that steroid glucuronide formation in patients with liver cirrhosis was reduced between 41% and 87% of normal. In this thesis, examination of the levels of the 17-OS in the plasma and in the urine of PCT patients revealed no abnormal elevations as in the hereditary hepatic porphyrias, but it is of interest to note that in 80% of the PCT patients examined, the total urinary excretion of the 17-OS was lower than the mean total urinary excretion of 17-OS of normals of the corresponding age group. In the plasma, the reverse was true, with 89% of the PCT patients whose plasma was examined having a greater level of total plasma 17-OS sulphates than the mean total plasma 17-OS level of normals of the same age group. These observations do suggest that there may be some impairment of steroid conjugation or excretion in the liver in these patients with PCT symptomata, resulting in slight increases in the concentrations of these steroids in the plasma or an even greater accumulation of the free steroids in the liver, where they might be partly responsible for the induction of the increased activity of ALA synthetase, which was found by Moore et al. (1972b).

Despite studies of structure-activity relationships within individual groups of chemically related compounds (Talman et al., 1957; Marks et al., 1965), the wide variety of chemical structures which can lead to induction of hepatic ALA synthetase has not so far permitted any unifying hypothesis concerning a

common chemical mechanism by which all these compounds act. Indeed, currently available information suggests that different groups of compounds may operate by different mechanisms. However, there is the possibility that the effects of the majority of these drugs are mediated through a change of steroid metabolism, or distribution, or an increase in the biological half-life of the steroid, resulting in an intracellular accumulation of an active inducing steroid in concentrations sufficient to impair repressor control of the synthesis of ALA.S.

Drugs and steroids are metabolised by the same cytochrome P-450-containing enzyme system in liver microsomes (Conney et al., 1968; Kupfer and Orrenius, 1970) and, from observations on the effect of castration, adrenalectomy and steroid hormones on enzyme activity (Kato and Gillette, 1965), it has been suggested that the levels of microsomal enzyme activity are controlled by the concentrations of circulating steroid hormones (Conney, 1967; Orrenius et al., 1968; Orrenius et al., 1969). The studies of Kato and Onoda (1970) on the regulation of the activity of drug oxidation in rat liver microsomes, suggested that androgens and oestrogens regulate drug hydroxylations, partly through the content of P-450 in the microsomes and principally through the capacity of P-450 to bind with the drugs. They reported that castration of male rats decreased the P-450 content of the microsomes, but this could be restored to normal by treatment with testosterone. It has also been shown that certain drugs and steroids, when

incubated together with liver microsomes, inhibit each other's oxidative metabolism in a competitive manner (Tephly and Mannering, 1968). Such competition could also occur in vivo; Orrenius and his colleagues (1968; 1969) have postulated that the induction of enzymes by drugs is mediated through changes taking place in an endogenous, steroid hormone controlled regulatory system as a result of interference with endogenous steroid metabolism. A similar regulatory mechanism might control the enzyme ALA synthetase, and potent porphyrinogenic drugs, like ALA, phenobarbital, DDC, which bind strongly to cytochrome P-450 (Jefcoate et al., 1969; De Matteis, 1970; Tschudy and Bonkowsky, 1972), might competitively inhibit steroid hydroxylation and thus enhance metabolism of steroids by pathways leading to active inducers (derepressors) of ALA synthetase. This inhibition of steroid hydroxylation by drugs, when incubated in vitro with liver microsomes in the presence of NADPH, has been demonstrated by Kuntzman et al. (1965) and by Schneidman et al. (1965). This hypothesis would explain the lack of ALA synthetase induction by AIA in adrenalectomised animals (Matsuoka et al., 1968; Moses et al., 1970) and the permissive effect of hydrocortisone on the induction of hepatic ALA synthetase in these animals (Marver et al., 1966c). An example of the disturbances in steroid metabolism which can be caused by these porphyrinogenic drugs was demonstrated by Southern et al. (1969), who reported that phenetharbital induces a moderate fall in the metabolic clearance and production rate of testosterone and a decrease

in the urinary excretion of androsterone, aetiocholanolone and the 11-oxy-17-OS. These changes are associated with a rise in the excretion of more polar metabolites of testosterone.

A similar interference with endogenous steroid metabolism may well explain the provocations of acute attacks in hereditary hepatic porphyria due to dieting (Welland et al., 1964; Felsher and Redeker, 1967; Knudsen et al., 1967; Eales, 1971) and the increased induction of porphyrins in experimental animals which have been starved (Marver et al., 1966a,b). There is a close association between carbohydrate metabolism and steroid turnover. Under conditions of fasting, there are changes in the supply of NADPH from the hexose monophosphate shunt, and a reduced hepatic content of pyridine nucleotide may contribute to the decreased rate of inactivation of the steroids by the various NADPH-dependent reducing enzymes. Hendrikx et al. (1968) reported that fasting subjects showed a fall in the urinary excretion of 17-OS, but this was accompanied by a modest increase in the plasma levels of DHA and androsterone sulphates. The hydrogenation of the steroids may be diminished by a low hepatic level of NADPH, owing to a fall in liver glycogen, which in turn leads to an insufficient supply of hydrogen from decrease in glucose-6-phosphate (Schriefers, 1967). Alternatively, starvation may be associated with a decreased activity of the various steroid-inactivating enzymes (Foy and Schneiden, 1962). Experiments in rats show that fasting results in a fall in hepatic steroid Δ^4 -reductase (Herbst et al., 1960) and 20-hydroxysteroid

dehydrogenase, both NADPH-dependent enzymes, and a reduced capacity of the liver to synthesise steroid glucuronides due to a limited formation of UDPGA (Schriefers et al., 1965a; 1966). This diminished supply of UDPGA, and hence glucuronide synthesis, is due to the lower levels of hepatic glycogen and glucose-6-phosphate, which fall to 10 - 20% of the control values on starvation. Like the hydrogenation, the capacity of the liver for formation of excretable steroid glucuronides may be brought back to values near normal in the case of fasting animals by the administration of glucose. Whether this type of mechanism, i.e. an increased capacity of the liver for hydrogenation and glucuronide conjugation of steroids, is responsible for the blocking of drug induced experimental porphyria in animals by high carbohydrate administration is not known. These findings in experimental porphyria were extended to patients with AIP (Welland et al., 1964; Felsher and Redeker, 1967) and VP (Perlroth et al., 1968; Eales, 1971) by the demonstration that a high intake of carbohydrate or protein, or both, can decrease porphyrin and precursor excretion. The data of Felsher and Redeker (1967) also indicates that a high carbohydrate diet can protect, some patients at least, against drug-induced attacks of AIP. The ability of glucose to block the induction of certain enzymes was originally demonstrated in micro-organisms by Magasanik (1961) and later this same effect, termed the 'glucose effect', was demonstrated to occur for enzymes in rat liver by Peraino and Pitot (1964). A number of these hepatic

enzymes which are repressed by glucose are involved in amino acid catabolism and gluconeogenesis, and in a number of systems this 'glucose effect' involves changes in the levels of insulin and glucagon and complex interaction with other hormones, particularly glucocorticoids (Jost et al., 1968; Hanoune et al., 1971). In patients suffering from hepatic porphyria, it is not known whether the beneficial effects of administration of a high protein or carbohydrate diet are mediated through this 'glucose effect' and hormonal influences, as suggested by Tschudy et al. (1964) and Marver et al. (1966b), or through a more direct mechanism resulting in increased capacity of the liver for detoxification and conjugation of inducing steroids.

These results and observations considered in this discussion indicate that the effects of many of the factors involved in the provocation and aggravation of the hepatic porphyrias may be mediated through interaction with an endogenous regulatory system of steroid metabolism in the liver. The liver is the centre of steroid metabolism and may be regarded as the antagonist to the endocrine glands, hypophysis and hypothalamus, which form a functional unit for the homeostatic regulation of the plasma levels of hormones. As mentioned previously, steroid metabolic activity of the liver is not constant but can be affected by a large variety of exogenous and endogenous factors which interfere with the normal metabolism and detoxification of the steroids. Some of these factors may well result in an increase in the activity of a specific steroid which induces the synthesis of ALA synthetase (in fact, there is

the possibility that the synthesis of this enzyme is under constant stimulation by an inducing steroid, in order to fulfil the requirement for haem under conditions of normal cellular activity). In the normal cell, this increase in activity of inducing steroid can be adequately dealt with by the corresponding increase in haem, resulting in greater feed-back repression of ALA synthetase or an increase in the haem-containing detoxifying enzymes. In the porphyric hepatic cell with its inherited defect of a partial block in the biosynthesis of haem (Kaufman and Marver, 1970; Meyer et al., 1971), the rise in the level of the inducer is not matched by a corresponding rise in haem production. With no increase in feed-back repression by haem or rise in the detoxifying enzyme system to combat this steroid inducer, further stimulation of the de novo synthesis of ALA synthetase will take place, accompanied by further increases in the steroid inducer if the original disturbance of steroid metabolism is still present. This cascading effect may have far reaching influences, causing disturbances in the function of the hypophysis, hypothalamus and steroid hormone-producing organs, culminating in the initiation of an acute attack. The relationship of these disturbances in steroid metabolism and resultant excess production of porphyrins and precursors to the multitude of clinical abnormalities, especially the neurological symptoms which have been observed in patients with hepatic porphyria, has not yet been established and awaits further investigation.

With respect to one clinical symptom, fever, which is sometimes present in a mild form during acute attacks of porphyria, it may be of potential interest to note that certain 5 β -H steroids which have strong porphyrinogenic action in liver cell culture (Granick and Kappas, 1967a) also have strong fever-producing action in man (Kappas and Palmer, 1963; 1965). Bondy et al. (1960) have reported steroid excretion patterns in a series of subjects suffering from fever in association with abdominal pain, joint pains and leucocytosis. They found elevated levels of urinary aetiocholanolone in a proportion, but in by no means all of such patients. These steroids with the 5 β -H configuration also have been shown to have haemolytic activity, and induce marked changes in the permeability of lysosomes releasing β -glucuronidase (Weissman, 1965; Weissman and Keiser, 1965). It remains possible that the steroids with the 5 β -H structure have similar disruptive action on nerve membrane, and perhaps in excess they might provide in part a possible explanation for the neurological disturbances and nerve fibre destruction sometimes observed in porphyria.

Lastly, this study of 17-OS and porphyrias raised one very important question concerning the regulation of haem biosynthesis in the cell. In animals, the control of haem biosynthesis appears to be primarily a control on the rate of biosynthesis of the enzyme ALA synthetase, and it is generally agreed that haem acts as a feed-back repressor of the synthesis of ALA synthetase. Since approximately 31 mg of haem are

formed daily in the liver, it seems likely that the haem biosynthetic pathway is under a constant stimulus. In vitro experimental work by several separate research groups indicates that certain steroids of physiological origin are capable of acting as derepressors, allowing the commencement of the transcription of the structural gene for the synthesis of ALA synthetase, and it seems very probable that in vivo a specific steroid may serve to maintain a certain rate of haem synthesis by inducing the synthesis of ALA synthetase. This specific steroid may well be DHA or a close metabolite of DHA, since the elevations of this steroid and its metabolites were the most striking abnormalities observed in this study of patients with hepatic porphyria. Furthermore, several general aspects of the behaviour of DHA and its sulphate have intrigued investigators for many years. DHA is secreted by the adrenal cortex in episodic bursts synchronously with hydrocortisone, and in amounts comparable to those of hydrocortisone. Likewise, DHAS is also secreted by the adrenal cortex in relatively large amounts and, apart from its role as a precursor in the placental production of oestrogen which is clearly established, no true hormonal effects have yet been described for DHAS. Compared to the free steroids and glucuronide conjugates, DHAS has a large half-life in the body, and thus a relative large quantity of sulphate conjugate of DHA is present at all times in the plasma, and there is evidence that this pool or reservoir of DHAS can maintain the plasma free DHA at a significant value above zero during periods when no free DHA is secreted by the adrenals. This is the

crucial difference between the behaviour of the two major adrenocortical secretory products, DHA and hydrocortisone, since the plasma concentration of hydrocortisone remains at values indistinguishable from zero for several hours in the late evening and early morning, whereas the concentration of DHA remains relatively high in relation to its peak values during the day (Rosenfeld et al., 1972). This seems strange when one compares the known effects of DHA (which are the inhibition of glucose-6-phosphate dehydrogenase and Δ^4 -5 α -reductase activity) to the far reaching effects of hydrocortisone on the body. This raises the intriguing possibility that DHA or a close metabolite may be as necessary to the human organism as hydrocortisone, and what could be more necessary than its action as a constant stimulus for the biosynthesis of haem?

REFERENCES

Abrahams, A., Gavey, C.J. & MacLagan, N.F. (1947).

Brit. med. J. 2,327.

Adlercreutz, H., Luukkainen, T. & Svanborg, A. (1967).

Ann. Med. Exp. Fenn. 45,277.

Adlercreutz, H., Kerstell, J., Svanborg, A. & Vihko, R.

(1968). Ann. Med. Exp. Fenn. 46,165.

Adlercreutz, H., Kerstell, J., Schaumann, K-O., Svanborg, A.

& Vihko, R. (1972) Eur. J. clin. Invest. 2,91.

Amoroso, E.C., Loosmore, R.M., Rimington, C. & Tooth, B.E.

(1957). Nature, 180,230.

Baron, J. & Tephly, T.R. (1969). Mol. Pharmacol. 5,10.

Batlle, A.M. del.C., Benson, A. & Rimington, C. (1965).

Biochem. J. 97,731.

Baulieu, E-E. (1960). J. clin. Endocr. 20,906.

Baulieu, E-E. (1962). J. clin. Endocr. 22,501.

Baulieu, E-E. (1963). Recent Progr. Hormone Res. 19,306.

Baulieu, E-E. (1965). In Hormonal Steroids (Martini, L.

& Pecile, A., Eds.) Vol.2, p.597. Academic Press:London & New York.

Baulieu, E-E. & Mauvais-Jarvis, P. (1964a).

J. biol. Chem. 239,1569.

Baulieu, E-E. & Mauvais-Jarvis, P. (1964b).

J. biol. Chem. 239,1578.

Baulieu, E-E., Corpécho, C., Dray, F., Emiliozzi, R.,

Lebeau, M-C., Mauvais-Jarvis, P. & Robel, P. (1965).

Recent Progr. Hormone Res. 21,411.

Baumstark, F. (1874). Pflüg. Arch. ges Physiol. 9,568.

Baxter, D.L. & Permowicz, S.E. (1967). Arch. Dermatol.

96,96.

Beattie, D.S. & Stuchell, R.N. (1970). Arch. Biochem.

Biophys. 139,291.

Becker, F.T. (1965). Arch. Dermatol. 92,252.

Bégué, J.A. (1965). C.R. Acad. Sci. (Paris), 260,3777.

Birke, G., Franksson, C. & Plantin, L-O. (1955).

Acta Endocrinol. 18,201.

Bloch, B. (1965). J. Obstet. Gynaecol. Brit. Commonw.

72,391.

Bock, K.W., Krauss, E. & Frohling, W. (1971).

Eur. J. Biochem. 23,366.

Bogorad, L. (1958a). J. biol. Chem. 233,501.

Bogorad, L. (1958b). J. biol. Chem. 233,510.

Bolgert, M., Canivet, J. & Le Sourd, M. (1953).

Sem. Hôp. Paris, 29,1587.

Bondy, P.K., Cohn, G.L. & Castiglione, C. (1960).

Trans. Ass. Amer. Phycns. 73,186.

Borst, B. & Königsdorffer, J.L. (1929). Untersuchung

über Porphyrurie mit besonderer Berücksichtigung der

Porphyria Congenita. Leipzig:Hirzel.

Braun, A. & Berman, J. (1959). Acta Univ. Carolinae Med.,

8,597.

Brown, E.G. (1958). Biochem. J. 70,313.

Brunsting, L.A. (1954). Arch. Dermatol. 70,551.

Bulbrook, R.D. (1972). J. natn. Cancer Inst. 48,1039.

Bulbrook, R.D. & Hayward, J.L. (1969). Lancet, 2,1033.

Burnham, B.F. & Lascelles, J. (1963). Biochem. J. 87,462.

Burstein, S. & Lieberman, S. (1958). J. biol. Chem.
233,331.

Burton, J.L., Loudon, N.B. & Wilson, A.T. (1967).
Lancet, 2,1326.

Bush, I.E., Swale, J. & Paterson, J. (1956).
Biochem. J. 62,16P.

Calvy, G.L., Jaruszewski, E.J. & Carrol, H.H. (1951).
Ann. intern. Med. 34,767.

Cam, C. (1959). Dirim (Istanbul), 34,11.

Cam, C. & Nigogosyan, G. (1963). J. Amer. med. Ass. 183,88.

Campbell, K. (1898). J. ment. Sci. 44,305.

Canivet, J. & Rimington, C. (1953). Biochem. J. 55,867.

Cawley, L.P., Musser, B.O. & Tretbar, H.A. (1967).
Amer. J. clin. Pathol. 48,216.

Cetingil, A.I. & Üzen, M.A. (1960). Blood, 16,1002.

Chapdelaine, A., MacDonald, P.C., Gonzalez, O., Gurpide, E.,
Vande Wiele, R.L. & Lieberman, S. (1965). J. clin. Endocr.
25,1569.

Charro-Salgado, A.L., Clarke, B.F., Shackleton, C.H.L.,
Duncan, L.J.P. & Mitchell, F.L. (1968). Lancet, 1,126.

Chatterton, R.T., Chatterton, A.J. & Hellman, L. (1970).
Endocrinology, 87,941.

Clayton, G.W., Bongiovanni, A.M. & Papadatos, C. (1955).
J. clin. Endocr. 15,693.

Cochrane, A.L. & Goldberg, A. (1968). Ann. hum. Genet. Lond.
32,195.

Cohn, G.L., Bondy, P.K. & Castiglione, C. (1961).
J. clin. Invest. 40,400.

Cohn, G.L. & Mulrow, P.J. (1963). J. clin. Invest. 42,64.

Coleman, R.R. (1948). J.S.C. med. Ass. 44,117.

Combes, B., Shibata, H., Adams, R., Mitchell, B.D. & Trammel, V.
(1963). J. clin. Invest. 42,1431.

Conference Discussion (1963). 'Proceedings of the International
Conference on the Porphyrrias', S. Afr. J. Lab. clin. Med. 9,301.

Connel, A.M., Cooper, J. & Redfearn, J.W. (1958).

Acta endocrinol. 27,179.

Conney, A.H. (1967). Pharmacol. Rev. 19,317.

Conney, A.H., Schneidman, K., Jacobson, M. & Kuntzman, R.

(1965). Ann. N.Y. Acad. Sci. 123,98.

Conney, A.H., Levin, W., Ikeda, M., Kuntzman, R., Cooper, D.Y.

& Rosenthal, O. (1968). J. biol. Chem. 243,3912.

Connon, J.J. & Turkington, V. (1968). Lancet, 2,263.

Conrad, S., Mahesh, V. & Herrman, W. (1961).

J. clin. Invest. 40,947.

Cookson, G.H. & Rimington, C. (1953). Nature, 171,875.

Cookson, G.H. & Rimington, C. (1954). Biochem. J. 57,476.

Copeman, P.W.M., Cripps, D.J. & Summerly, R. (1966).

Brit. med. J. 1,461.

Cornford, P. (1964). Biochem. J. 91,64.

Correl, H.L., Peters, B.J. & Murphy, F.D. (1942).

Urol. cutan. Rev. 46,341.

- Cox, R.F. & Mathias, A.P. (1969). Biochem. J. 115,777.
- Coyle, M.G. & Brown, J.B. (1963). J. Obstet. Gynaecol.
Brit. Commonw. 70,225.
- Cronholm, T. & Sjövall, J. (1970). Eur. J. Biochem. 13,124.
- Dean, G. (1953). Brit. med. J. 2,1291.
- Dean, G. (1963). S. Afr. J. Lab. clin. Med. 9,145.
- Dean, G. (1965). S. Afr. J. Lab. clin. Med. 13,278.
- Dean, G. & Barnes, H.D. (1955). Brit. med. J. 2,89.
- Dean, G. & Barnes, H.D. (1959). S. Afr. med. J. 33,274.
- Dean, G., Kranmer, S. & Lamb, P. (1967).
S. Afr. med. J. 43,138.
- Degos, R., Touraine, R., Kalis, B., Delort, J. & Bonvalet, D.
(1969). Ann. Dermatol. Syphiligr. (Paris), 96,5.
- De Neve, L. & Vermeulen, A. (1965). J. Endocrinol. 32,295.
- Denny-Brown, D. & Sciarra, D. (1945). Brain, 68,1.

De Matteis, F. (1964). Biochim. biophys. Acta, 82,641.

De Matteis, F. (1966).¹ In Experimental Study of the Effects of Drugs on the Liver, p.156. Excerpta Medica Foundation: Amsterdam.

De Matteis, F. (1970). F.E.B.S. Lett. 6,343.

De Matteis, F., Prior, B.E. & Rimington, C. (1961).
Nature, 191,363.

De Moor, P., Osinski, P., Deckx, R. & Steeno, O. (1962).
Clin. chim. Acta, 7,475.

Dingman, J.F., Lim, N.Y. (1963). J. Amer. med. Ass. 186,316.

Dobrschansky, M. (1906). Wien. med. Presse, 47,2145.

Doss, M. & Kaltepoth, B. (1971). 'Proceedings of the International Conference on Porphyrin Metablism and the Porphyrrias' (Special Issue: S. Afr. J. Lab. clin. Med., 25 Sept., p.73).

Dorfman, R.I. (1969). In Methods in Hormone Research (Ed. Dorfman, R.I.), 2nd Edn., Vol. 2, p.151.
Academic Press:New York.

Dorfman, R.I. & Sharma, D.C. (1965). Steroids, 6,229.

Dowdle, E.B., Mustard, P. & Eales, L. (1967).

S. Afr. med. J. 41,1093.

Dowdle, E.B., Mustard, P., Spong, N. & Eales, L. (1968).

Clin. Sci. 34,233.

Drayer, N.M. & Lieberman, S. (1965). Biochem. biophys.

Res. Comm. 18,126.

Dresel, E.I.B. & Falk, J.E. (1953). Nature, 172,1185.

Drews, J. & Brawerman, G. (1967). J. biol. Chem. 242,801.

Dutton, G.J. (1966). In Glucuronic Acid, Free and Combined
(Ed., Dutton, G.J.), p.186. Academic Press: New York & London.

Eales, L. (1961). Ann. Rev. Med. 12,251.

Eales, L. (1963). S. Afr. J. Lab. clin. Med. 9,151.

Eales, L. (1963a). S. Afr. J. Lab. clin. Med. 9,261.

Eales, L. (1971). 'Proceedings of the International
Conference on Porphyrin Metabolism and the Porphyrrias'
(Special Issue: S. Afr. J. Lab. clin. Med., 25th Sept., p.120).

Edwards, R.W.H., Kellie, A.E. & Wade, A.P. (1953).

Mem. Soc. Endocrinol. 2,53.

Eisalo, A., Jarvinen, P.E. & Luukkainen, T. (1964).

Brit. med. J. 1,426.

Eliaser, M. & Kondo, B.O. (1942). Amer. Heart J. 24,696.

Falk, J.E., Porra, R.J., Brown, A., Moss, F. & Larminie, M.E.

(1959). Nature, 184,1217.

Fehér, T. (1966). Clin. chim. Acta, 14,91.

Fehér, T., Koref, O. & Kazik, M.H. (1966).

Acta endocrinol (Kbh.), 51,429.

Felsher, B.F. & Redeker, A.G. (1966). Medicine, 45,575.

Felsher, B.F. & Redeker, A.G. (1967). Medicine, 46,217.

Ferguson, J.C., Beattie, A.D., McAlpine, S.G. & Conway, H.

(1970). Postgrad. med. J. 46,717.

Fischer, H. (1915a). Hoppe-Seylers Z. physiol. Chem.

95,34.

Fischer, H. (1915b). Hoppe-Seylers Z. physiol. Chem.

96,148.

Fischer, H. (1927). Über Porphyrine und ihre Synthesen.

Bericht. D. Deutsch. Chem. Gessellech. 17,2611.

Fischer, H. (1930). Über Blut-, Blatt- und Gallen-Farbstoffe.
Oppenheimer's Handb. d. Biochem. des Menschen und d. Tiere,
Zweite Auflage, Ergänzungsband, p.72.

Fischer, H. & Duesberg, R. (1932). Arch. exptl. Path.
Pharmacol. 166,95.

Fischer, H. & Orth, H. (1937). In Chemie des Pyrrols,
Leipzig:Axademische Verlagsgesellschaft.

Fischer, H., Hilmer, H., Linder, F. & Pützer, B. (1925).
Z. Physiol. Chem. 150,44.

Fishman, W.H. (1963). In Methods of Enzymatic Analysis
(Ed. Bergmeyer, H.U.), p.867. Academic Press:London.

Forchielli, E. & Dorfman, R.I. (1956). J. biol. Chem. 223,443.

Fourie, P.J.J. (1936). Onderstepoort J. vet. Sci. 7,535.

Fourie, P.J.J. (1939). Onderstepoort J. vet. Sci. 13,383.

Foy, J.M. & Schnieden, H. (1962). J. Endocr. 24,403.

French, F.M. & Thonger, E. (1966). Clin. Sci. 31,337.

Frost, J.W., Dryer, R.L. & Kohlstaedt, K.G. (1951).
J. lab. clin. Med. 38,523.

Gajdos, A. & Gajdos, M. (1969). In Porphyries et Porphyries, p.154. Masson:Paris.

Gallagher, C.H. (1960). Biochem. J. 74,38.

Gallagher, C.H., Mueller, M.N. & Kappas, A. (1966). Medicine, 45,471.

Garrod, A.E. (1923). In Inborn Errors of Metabolism, 2nd Edn. (Ed. Frowde). Hodder & Stoughton:London.

Gibson, K.D., Neuberger, A. & Scott, J.J. (1955). Biochem. J. 61,618.

Gillette, J.R. & Gram, J.E. (1969). In Microsomes and Drug Oxidations (Eds. Gillette, J.R., Conney, A.H., Cosmides, G.J., Estabrook, R.W., Fouts, J.R. & Mannering, G.J.), p.133. Academic Press:New York & London.

Gillette, P.N., Bradlow, H.L., Gallagher, T.F. & Kappas, A. (1970). J. clin. Invest. 49,34a.

Goldberg, A. (1954). Biochem. J. 57,55.

Goldberg, A. (1959). Quart. J. Med. 28,183.

Goldberg, A. (1966). In Modern Trends in Dermatology (Ed. McKenna, R.M.B.). London.

Goldberg, A. (1971). In Recent Advances in Haematology, p.302. (Eds. Goldberg, A. & Bain, M.C.). Churchill Livingstone:Edinburgh and London.

Goldberg, A. & Rimington, C. (1962). In Diseases of Porphyrin Metabolism. Thomas:Springfield, Illinois.

Goldberg, A., MacDonald, A.C. & Rimington, C. (1952).

Brit. med. J. 2,1174.

Goldberg, A., Ashenbrucker, H., Cartwright, G.E. &

Wintrobe, M.M. (1956). Blood, 2,821.

Goldberg, A., Rimington, C. & Lochhead, A.C. (1967).

Lancet, 1,632.

Goldberg, A., Moore, M.R., Beattie, A.D., Hall, P.E.,

McCallum, J. & Grant, J.K. (1969). Lancet, 1,115.

Goldswain, P.R. & Eales, L. (1971). 'Proceedings of the

International Conference on Porphyrin Metabolism and the

Porphyrias' (Special Issue: S. Afr. J. Lab. clin. Med.,

25 Sept., p.111).

Goldzieher, J.W. & Beering, S.C. (1969). J. clin. Endocr.

29,171.

Gordon, A.S., Zanjani, E.D., Levere, R.D. & Kappas, A. (1970).

Proc. natn. Acad. Sci. 65,919.

Gorschein, D. & Gardner, F.M. (1970). Proc. natn. Acad. Sci.

65,564.

Granick, S. (1966). J. biol. Chem. 241,1359.

Granick, S. & Kappas, A. (1967a). Proc. natn. Acad. Sci.
57,1463.

Granick, S. & Kappas, A. (1967b). J. biol. Chem. 20,4587.

Granick, S. & Levere, R. (1968). Proc. 12th Int. Soc.
Haemat., p.274. Grune & Sutton: New York.

Granick, S. & Sassa, S. (1971). In Metabolic Regulation
(Ed. Vogel, H.J.). Academic Press:New York & London.

Granick, S. & Urata, G. (1963). J. biol. Chem. 238,821.

Gray, A.M.H. (1925). Quart. J. Med. 19,381.

Gray, C.H. (1970). In Biochemical Disorders in Human Disease
(Eds. Thompson, R.H.S., Wooton, I.D.P.J. & Churchill, P.)
3rd Edn., p.215. London.

Gray, C.H., Nicholson, D.C., Kulczycka, A., Magnus, I.A. &
Rimington, C. (1964). Clin. Sci. 26,7.

Griffiths, K., Grant, J.K. & Symington, T. (1963).
J. clin. Endocr. 23,776.

Grossfield, E. (1951). Brit. med. J. 1,1240.

Grundy, S.M., Ahrens, E.H. & Miettinén, T.A. (1965).

J. Lipid Res. 6,397.

Günther, H. (1911). Dtsch. Arch. klin. Med. 105,89.

Günther, H. (1922). Ergeben. allg. Path. path. Anat.
20,608.

Haeger-Aronsen, B. (1963a). Amer. J. Med. 35,450.

Haeger-Aronsen, B. (1963b). S. Afr. J. Lab. clin. Med.
9,288.

Haeger-Aronsen, B. & Krook, G. (1965). Acta med. scand.
445,48.

Haeger-Aronsen, B., Stathers, G. & Swahn, G. (1968).
Ann. intern. Med. 69,221.

Hamburger, C. (1948). Acta endocrinol. 1,19.

Hammarsten, O. (1891). Uppsala Läk-För. Förh. 26,259.

Hammarsten, O. (1892). Skand. Arch. Physiol. 3,318.

Hamnström, B., Haeger-Aronsen, B., Waldenström, J. Hysing, B.
& Molander, J. (1967). Brit. med. J. 4,449.

Hanoune, J., Chambaut, A-M. & Josipowicz, A. (1971).

Biochim. biophys. Acta, 244,338.

Harley, V. (1890). Brit. med. J. 2,1169.

Harris, H. (1970). In The Principles of Human Biochemical Genetics, p.211. American Elsevier Publishing Company:New York.

Hayashi, N., Yoda, B. & Kikuchi, G. (1968). J. Biochem. 63,446.

Heilmeyer, L. & Clotten, R. (1964). Germ. med. Mon. 9,353.

Heilmeyer, L. & Clotten, R. (1969). Klin. Wschr. 47,71.

Hellman, L., Bradlow, H.L., Zumoff, B., Fukushima, D.K. & Gallagher, T.F. (1959). J. clin. Endocr. 19,936.

Hellman, E.S., Tschudy, D.P. & Bartter, F.C. (1962). Amer. J. Med. 32,734.

Hendrikx, A., Heyns, W., De Moor, P. (1968). J. clin. Endocr. 28,1525.

Hendrikx, A., Heyns, W., Steeno, O. & De Moor, P. (1965). In Androgens in Normal and Pathological Conditions, p.63 (Eds. Vermeulen, A. & Exley, D.). Excerpta Medica Foundation: London.

Herbst, A.L., Yates, F.E., Glenister, D.W. & Urquhart, J.
(1960). Endocrinology, 67,222.

Heyns, W. & De Moor, P. (1965). In Androgens in Normal
and Pathological Conditions, p.42. (Eds. Vermeulen A. &
Exley, D.). Excerpta Medica Foundation:London.

Holti, G., Rimington, C., Tate, B.C. & Thomas, G. (1958).
Quart. J. Med. N.S. 27,1.

Hoppe-Seyler, F. (1871). Medizinische-chemische Unter-
suchungen, Berlin, 1-4,528.

Horton, R. (1966). Recent Progr. Hormone Res. 22,279.

Horton, R. & Tait, J.F. (1966). In Androgens in Normal
and Pathological Conditions, p.199. (Eds. Vermeulen, A.
& Exley, D.). Excerpta Medica Foundation:London.

Horton, R., Romanoff, E. & Walker, J. (1966). J. clin.
Endocr. 26,1267.

Hunter, J.A.A., Khan, S.A., Hope, E., Beattie, A.D.,
Beveridge, G.W., Smith, A.W.M. & Goldberg, A. (1971).
Brit. J. Dermatol. 84,301.

Hurley, M.J. & English, R.S. (1963). Arch. Dermatol.
88,233.

Ichii, S. & Yago, N. (1969). J. Biochem. 65,597.

Incefy, G.S. & Kappas, A. (1971). F.E.B.S. Lett. 15,153.

Ingle, D.J. (1954). Acta endocrinol. 17,172.

Ismail, A.A.A. & Harkness, R.A. (1966). J. Endocr. 34,17.

Isselbacher, K.J. & Krane, S.M. (1961). J. biol. Chem.
236,2394.

Jacob, F. & Monod, J. (1961). J. molec. Biol. 3,318.

Jänne, O. (1971). Acta endocrinol. 67,316.

Jänne, O., Vihko, R., Sjövall, J. & Sjövall, K. (1969).
Clin. chim. Acta, 23,405.

Janousek, V. (1970). Trans. St. John's Hosp. derm. Soc.
(London), 56,14.

Jefcoate, C.R.E., Gaylor, J.L. & Calabrese, R.L. (1969).
Biochemistry, 8,3455.

Johnsen, S.G. (1968). Acta endocrinol. 57,595.

Jost, J-P., Khairallan, E.A. & Pitot, H.C. (1968).

J. biol. Chem. 243,3057.

Jutzler, G.A., Neuheisel, S. & Schmid, P. (1964).

Ger. med. Monthly, 9,402.

Kaplowitz, N., Javitt, N. & Harber, C.L. (1968).

N. Engl. J. Med. 278,1077.

Kappas, A. & Granick, S. (1968). J. biol. Chem. 243,346.

Kappas, A. & Palmer, R.H. (1963). Pharmacol. Rev. 15,123.

Kappas, A. & Palmer, R.H. (1965). In Methods in Hormone Research, Vol. 4, p.1. (Ed. Dorfman, R.I.). Academic Press:New York.

Kappas, A., Levere, R.D. & Granick, S. (1968). 'Seminars Haematol. 5,323.

Kappas, A., Song, C.S., Levere, R.D., Sachson, R.A. & Granick, S. (1968). Proc. natn. Acad. Sci. 61,509.

Kappas, A., Song, C.S., Sassa, S., Levere, R.D. & Granick, S. (1969). Proc. natn. Acad. Sci. 64,557.

Kappas, A., Bradlow, H.L., Gillette, P.N. & Gallagher, T.P. (1971). Ann. N.Y. Acad. Sci. 179,611.

Karibian, D. & London, I.M. (1965). Biochem. biophys. Res. Commun. 18,243.

Kato, R. & Gillette, J.R. (1965). J. Pharmacol. exp. Ther. 150,285.

Kato, R. & Onoda, K. (1970). Biochem. Pharmacol. 19,1649.

Kaufman, L. & Marver, H. (1970). N. Engl. J. Med. 283,954.

Kaufman, B.M., Vickers, H.R., Rayne, J. & Ryan, T.J. (1967). Brit. J. Dermatol. 79,210.

Kelenyi, G., Arato, G., Buda, V. & Orban, S. (1960). Lancet, 1,434.

Kellie, A.E. & Smith, E.R. (1957). Biochem. J. 66,490.

Keutman, E.H. & Mason, W.B. (1967). J. clin. Endocr. 27,406.

Kirschner, M.A. & Lipsett, M.B. (1964). Acta endocrinol. 46,207.

Kirschner, M.A., Lipsett, M.B. & Wilson, H. (1963). Acta endocrinol. 43,387.

Kirschner, M.A., Lipsett, M.B. & Collins, D.R. (1965). J. clin. Invest. 44,657.

Klopper, A. (1957). J. Obstet. Gynaecol. Brit. Commonw.
64,504.

Knudsen, K.B., Sparberg, M. & Lecocq, F. (1967).
N. Engl. J. Med. 277,350.

Koskelo, P. & Toivonen, I. (1968a). Clin. chim. Acta,
21,291.

Koskelo, P. & Toivonen, I. (1968b). Acta obstet. gynecol.
Scand. 47,292.

Koskelo, P., Eisalo, A. & Toivonen, I. (1966).
Brit. med. J. 1,652.

Kottra, J. & Kappas, A. (1967). Ann. Rev. Med. 18,325.

Kramer, S., Viljoen, E., Meyer, A.M. & Metz, J. (1965).
Brit. J. Haematol. 11,666.

Kuntzman, R., Jacobson, M., Schneidman, K. & Conney, A.H.
(1964). J. Pharmacol. exp. Ther. 146,280.

Kumari, G.L., Collins, W.P. & Sommerville, I.F. (1969).
J. Chromatogr. 41,22.

Kupfer, D. & Orrenius, S. (1970). Eur. J. Biochem. 14,319.

Kurashima, Y., Hayashi, N. & Kikuchi, G. (1970).

J. Biochem. 67,863.

Laatikainen, T. & Vihko, R. (1968). J. clin. Endocr.

28,1356.

Laatikainen, T. & Vihko, R. (1969). Eur. J. Biochem.

10,165.

Laatikainen, T. & Vihko, R. (1971). J. Steroid Biochem.

2,173.

Laatikainen, T., Laitinen, E.A. & Vihko, R. (1969).

J. clin. Endocr. 29,219.

Labbe, R.F. (1967). Lancet, 1,1361.

Labbe, R.F. & Hubbard, N. (1960). Biochim. biophys. Acta,

41,185.

Laidlaw, P.P. (1904). J. Physiol. 31,464.

Lascelles, J. (1964). In Tetrapyrrole Biosynthesis and its Regulation. N.A. Bengamin, Inc.:New York.

Lathe, G.H. & Walker, M. (1958). Quart. J. exp. Physiol.

43,257.

Lau, H.L. (1966). J. Gas Chromatogr. 4,136.

Levere, R.D. (1966). Blood, 28,569.

Levere, R.D. & Granick, S. (1965). Proc. natn. Acad. Sci.
54,134.

Levere, R.D. & Mizoguchi, H. (1971). Clin. Res. 19,565.

Levere, R.D., Kappas, A. & Granick, S. (1967).
Proc. natn. Acad. Sci. 58,985.

Levit, E.J., Nodine, J.H. & Perloff, W.H. (1957).
Amer. J. Med. 22,831.

Linaz, S., Solomon, M.L. & Figge, F.H.J. (1947).
J. Amer. med. Ass. 133,105.

Linder, G.C. (1947). Lancet, 2,649.

Lockwood, W.H. & Benson, A. (1960). Biochem. J. 75,372.

Loomis, W.F. & Magasanik, B. (1964). J. molec. Biol.
8,417.

Lopez-S, A. & Krehl, W.A. (1967). Lancet, 2,485.

Loraine, J.A. & Bell, E.T. (1971). In Hormone Assays and their Clinical Application, 3rd Edn. E. & S. Livingstone: Edinburgh & London.

Ludwig, G.D. & Goldberg, M. (1963). Ann. N.Y. Acad. Sci. 104,710.

MacDonald, P.C., Vande Wiele, R.L. & Lieberman, S. (1962). J. clin. Endocr. 22,1222.

MacDonald, P.C., Chapdelaine, A., Gonzalez, O., Gurpide, E., Vande Wiele, R.L. & Lieberman, S. (1965). J. clin. Endocr. 25,1557.

MacGregor, A.G., Nicholas, R.E.H. & Rimington, C. (1952). Arch. intern. Med. 90,483.

McGuire, J.S. & Tomkins, G.M. (1958). Nature, 182,261.

McGuire, J.S. & Tomkins, G.M. (1959). J. biol. Chem. 234,791.

McIntyre, N., Pearson, A.J.G., Graske, S., West, G.M.L., Moore, M.R., Beattie, A.D., Paxton, J.W. & Goldberg, A. (1971). Lancet, 1,560.

McKenzie, A.W. & Acharya, U. (1972). Brit. J. Dermatol. 86,453.

Magasanik, B. (1961). Symp. on Quant. Biol. 26,249.

Magnus, I.A. (1968). Seminars Haematol. 4,380.

Magnus, I.A., Jarret, A., Prankerd, T.A.J. & Rimington, C.
(1961). Lancet, 2,448.

Marks, G.S., Hunter, E.G., Turner, U.K. & Schneek, D. (1965).
Biochem. Pharmacol. 14,1077.

Marshall, W.J. (1971). Biochem. Pharmacol. 20,1723.

Marshall, W.J. & MacLean, A.E.M. (1969). Biochem. J. 115,27P.

Martin, M.M., Mintz, D.H. & Tamagaki, H. (1963). J. clin.
Endocr. 23,242.

Marver, H.S. (1969). In Microsomes and Drug Oxidations,
p.495. Academic Press:New York.

Marver, H.S., Tschudy, D.P., Perlroth, M.G. & Collins, A.
(1966a). J. biol. Chem. 241,2803.

Marver, H.S., Collins, A., Tschudy, D.P. & Rechcigl, M.
(1966b). J. biol. Chem. 241,4323.

Marver, H.S., Collins, A. & Tschudy, D.P. (1966c).
Biochem. J. 99,31c.

Marver, H.S., Schmid, R. & Schützel, H. (1968).

Biochem. biophys. Res. Commun. 33,969.

Mason, J.W. (1968). Psychosom. Med. 5,576.

Masuya, T. (1969). Acta haematol. jap. 32,519.

Matsuoka, T., Yoda, B. & Kikuchi, G. (1968).

Arch. Biochem. Biophys. 126,530.

Mauzerall, D. & Granick, S. (1956). J. biol. Chem.

219,435.

Mauzerall, D. & Granick, S. (1958). J. biol. Chem.

232,1141.

Meyer, U.A. & Marver, H.S. (1971). Science, 171,64.

Meyer, U.A., Strand, L.J., Doss, M., Rees, A.C. & Marver, H.S.

(1972). N. Engl. J. Med. 286,1277.

Migeon, C.J. (1956). J. biol. Chem. 218,941.

Migeon, C.J. & Plager, J.E. (1954a). J. biol. Chem. 209,767.

Migeon, C.J. & Plager, J.E. (1954b). Recent Progr. Hormone

Res. 9,235.

- Migeon, C.J., Keller, A.R., Lawrence, B. & Shepard, T.H.
(1957). J. clin. Endocr. 17,1051.
- Mikhail, G. (1970). Gynecol. Invest. 1,5.
- Mills, E.S. & Topper, Y.J. (1969). Science, 165,1127.
- Miyagi, K. (1967). J. Kyushu Haematol. Soc. 17,397.
- Miyagi, K. (1970). J. Kyushu Haematol. Soc. 20,190.
- Moore, M.R. (1970). Ph.D. Thesis, University of Glasgow.
- Moore, M.R. (1972). Personal Communication.
- Moore, M.R., Battistini, V., Beattie, A.D. & Goldberg, A.
(1970). Biochem. Pharmacol. 19,751.
- Moore, M.R., Thompson, G.G. & Goldberg, A. (1972a).
Clin. Sci. 43,299.
- Moore, M.R., Barnardo, D., Magnus, I.A., Turnbull, A.L.,
Beattie, A.D. & Goldberg, A. (1972b). Lancet, 1,97.
- Moses, H.L., Stein, J.A. & Tschudy, D.P. (1970).
Lab. Invest. 22,432.
- Moser, H.W., Moser, A.B. & Orr, J.C. (1966).
Biochim. biophys. Acta, 116,146.

Mueller, N.M. & Kappas, A. (1964). Trans. Ass. Amer. Physicians, 77,248.

Mulder (1844). J. prakt. Chem. 32,186.

Nakao, K., Wada, O., Kitamura, T., Vono, K. & Urata, G. (1966). Nature, 210,838.

Necheles, T.F. & Rai, U.S. (1969). Blood, 34,380.

Neuberger, A. (1968). Proc. roy. Soc. Med. 61,191.

Nielson, D.R. & Nielson, R.P. (1958). West. J. Surg. 66,133.

Nocke, W. & Breuer, H. (1963). Acta endocrinol. 44,47.

Nyström, E. & Sjövall, J. (1965). Anal. Biochem. 12,235.

Ockner, P.K. & Schmid, R. (1961). Nature, 189,499.

O'Dwyer, J.P. (1955). J. Obstet. Gynaecol. Brit. Commonw. 64,437.

Oertel, G.W. & Kaiser, E. (1962). Clin. chim. Acta, 7,221.

O'Kelly, D. (1968). Ph.D. Thesis, Univ. of Glasgow.

Onisawa, J. & Labbé, R.F. (1963). J. biol. Chem. 238,724.

Orrenius, S., Gnosspelius, Y., Das, M.L. & Ernster, L.
(1968). In Symposium on the Structure and Function of the
Endoplasmic Reticulum in Animal Cells. (Ed. Campbell, P.N.).
Universitetsforlaget:Oslo.

Orrenius, S., Das, M.L. & Gnosspelius, Y. (1969).
In Microsomes and Drug Oxidations. (Eds. J.R. Gillette, et al.).
Academic Press:New York.

Pathak, M.A. & Burnett, J.W. (1964). J. invest. Dermatol.
43,119.

Patti, A.A. & Stein, A.A. (1964). In Steroid Analysis by
Gas-Liquid-Chromatography, p.36. C.C. Thomas:Springfield,
Illinois.

Peraino, C. & Pitot, H.C. (1964). J. biol. Chem. 239,4308.

Perlroth, M.G., Marver, H.S. & Tschudy, D.P. (1965).
J. Amer. med. Ass. 194,1037.

Perlroth, M.G., Tschudy, D.P., Marver, H.S., Berard, C.W.,
Zeigel, R.F., Rechcigl, M. & Collins, A. (1966).
Amer. J. Med. 41,149.

Perlroth, M.G., Tschudy, D.P., Ratner, A., Spaur, W. &
Redeker, A. (1968). Metabolism, 17,571.

Peterson, R.E. & Wyngaarden, J.B. (1956). J. clin. Invest.
35,522.

Petrie, S.J. & Mooney, J.P. (1962). Amer. J. Obstet.
Gynecol. 83,264.

Pincus, G. (1947). Recent Progr. Hormone Res. 1,123.

Pincus, G. & Hoagland, H. (1943). J. Aviat. Med. 14,173.

Plager, J.E. (1965). J. clin. Invest. 14,1234.

Porra, R.J. & Jones, O.T.G. (1963). Biochem. J. 87,181.

Prato, V. & Zina, G. (1965). In Proceedings of the Inter-
national Symposium on the Normal and Pathologic Metabolism
of Porphyrins, p.82. Saint Vincent:Edizioni Minerva Media.

Prentice, R.T.W. & Goldberg, A. (1967). Brit. J. Dermatol.
79,682.

Prentice, R.T.W. & Goldberg, A. (1969). Brit. J. Dermatol.
81,690.

Prunty, F.T.G. (1946). Arch. intern. Med. 77,623.

Prunty, F.T.G. (1949). J. clin. Invest. 28,690.

Rao, L.G.S. (1972). Nature, 235,220.

Rancourt, M.W. & Litwack, G. (1968). Exp. Cell Res.
51,413.

Ranking, J.E. & Pardington, G.L. (1890). Lancet, 2,607.

Rawlings, E.E. (1950). Brit. med. J. 1,549.

Redeker, A.G. (1963). S. Afr. J. Lab. clin. Med. 9,302.

Redeker, A.G. (1964). Lancet, 2,1178.

Redeker, A.G. & Bronow, R.S. (1964). Arch. Dermatol.
89,104.

Redeker, A.G. & Sterling, R.E. (1968). Arch. intern. Med.
121,446.

Redeker, A.G., Bronow, R.S. & Sterling, R.E. (1963).
S. Afr. Lab. clin. Med. 9,235.

Reidenberg, L. (1955). Amer. J. Obstet. Gynecol. 69,879.

Rifkind, A.B., Gillette, P.N., Song, C.S., Kappas, A. (1970).

J. clin. Endocr. 30,330.

Rimington, C. (1956). Brit. med. J. 2,189.

Rimington, C. (1958). Rev. Pure Appl. Chem. 8,129.

Rimington, C. (1959a). Brit. med. J. 15,19.

Rimington, C. (1959b). In Handbuch des Gesamten Hämatologie,
2 aufl., p.145. (Eds. Heilmeyer, L. & Hittmair, A.). Munich.

Rimington, C. (1961). Association of Clinical Pathologists,
Broadsheet No. 36.

Rimington, C. (1963). Ann. N.Y. Acad. Sci. 104,666.

Rimington, C. (1966). Relazione al 7 Congresso Internazionale
di Patologia Clinica, Rome.

Rimington, C. & De Matteis, F. (1965). Lancet, 1,270.

Rimington, C., Lockwood, W.H. & Belcher, R.V. (1968).
Clin. Sci. 35,211.

Rivera, R., Dorfman, R.I. & Forchielli, E. (1967).
Acta endocrinol. 54,37.

Roberts, K., Vande Wiele, R.L. & Lieberman, S. (1961).
J. biol. Chem. 236,2213.

Romeo, G., Glenn, B.L. & Levin, E.Y. (1970). Biochem.
Genet. 4,6.

Rose, J.A., Hellman, E.S. & Tschudy, D.P. (1961).

Metabolism, 10,514.

Rosenfeld, R.S., Hellman, L. & Gallagher, T.F. (1972).

J. clin. Endocr. 35,187.

Rovinsky, J.J. & Guttmacher, A.F. (1965). In Medical, Surgical and Gynecological Complications of Pregnancy, p.784.
Williams & Williams: Baltimore.

Roy, A.B. (1956). Biochem. J. 62,41.

Ruokonen, A., Laatikainen, T., Laitinen, E.A. & Vihko, R.
(1972). Biochemistry, 11,1411.

Sandberg, E., Gurside, E. & Lieberman, S. (1964).

Biochemistry, 3,1256.

Sano, S. & Granick, S. (1961). J. biol. Chem. 236,1173.

Sardesai, V.M., Weissman, E.B., Locke, E.R. & Orten, J.M.

(1970). Biochem. Med. 4,440.

Sassa, S. & Granick, S. (1970). Proc. natn. Acad. Sci.

67,517.

Scherer, J. (1841). Ann. D. Chem. u. Pharmacol. 40,1.

Schmid, R. (1960). N. Engl. J. Med. 263,397.

Schmid, R. (1966). In The Porphyrrias, the Metabolic Basis of Hereditary Disease, p.813. (Eds. Stanbury, J.B., Wyngaarden, J.B. & Fredrickson, D.S.), 2nd Edn. McGraw-Hill Book Co.:New York.

Schmid, R., Schwartz, S. & Watson, C.J. (1954). Arch. intern. Med. 93,167.

Schmid, R., Schwartz, S. & Sunberg, R.D. (1955a). J. Haematol. 10,5.

Schmid, R., Figen, J.F. & Schwartz, S. (1955b). J. biol. Chem. 217,263.

Schmid, R., Marver, H.S. & Hammaker, L. (1966). Biochem. biophys. Res. Commun. 24,319.

Schneck, D.W., Tyrrell, L.J. & Marks, G.S. (1971). Biochem. Pharmacol. 20,2999.

Schneider, J.J. & Lewbart, M.L. (1956). J. biol. Chem. 222,787.

Schneidman, K., Jacobson, M. & Kuntzman, R. (1965).

Ann. N.Y. Acad. Sci. 123,98.

Scholnick, P., Hammaker, L.E. & Marver, H.S. (1969).

Proc. natn. Acad. Sci. 63,65.

Schothorst, A.A. Van Steveninck, J., Went, L.M. & Suurmond, D.

(1970). Clin. chim. Acta, 28,41.

Schriefers, H. (1965). Acta endocrinol. 50,25.

Schriefers, H. (1967). Vitam. Horm. 25,271.

Schriefers, H. (1969). In Advances in the Biosciences 2,

p.69. (Ed. Raspé, G). Pergamon Press:London.

Schriefers, H., Keck, B. & Otto, M. (1965).

Acta endocrinol. 50,25.

Schriefers, H., Ghraf, R. & Pohl, F. (1966).

Z. Physiol. Chem. 344,25.

Schück, O. & Berman, J. (1956). Čas. Lék, česk. 95,125.

Schulman, M.P. & Richert, D.A. (1956). Fed. Proc. 15,349.

Schultz, J.H. (1874). In Ein Fall von Pemphigus Leprosus, Complicirt Durch Lepa Visceralis. Inaug Dissertation: Griefswald.

Schumn, O. (1924). Hoppe-Seylers Z. Physiol. Chem. 139,219.

Schwartz, S., Johnson, J.A., Stephenson, B.D., Anderson, A.S., Edmondson, P.R. & Fusaro, R.M. (1971). J. Lab. clin. Med. 78,411.

Shanley, B.C., Zail, S.S. & Joubert, S.M. (1969). Personal Communication.

Shearman, R.P. (1959). J. Obstet. Gynaecol. Brit. Commonw. 66,1.

Shemin, D. (1956a). Harvey Lectures Ser. 50,258.

Shemin, D. (1956b). In Currents in Biochemical Research, p.518. (Ed. Green, D.E.). Interscience:New York.

Shemin, D. (1970). Naturwissenschaften, 57,185.

Shemin, D. & Kumin, S. (1952). J. biol. Chem. 198,827.

Short, R.V. (1960). Biochem. Soc. Symp. 18,59.

Silverberg, A., Rizzo, F. & Kreiger, D.T. (1968).

J. clin. Endocr. 28,1661.

Sirett, N.E. & Gibbs, F.P. (1969). Endocrinology, 85,355.

Slaunwhite, W.R., Burgett, M.J. & Sandberg, A.A. (1967).

J. clin. Endocr. 27,663.

Slaunwhite, W.R., Neely, L. & Sandberg, A.A. (1964).

Steroids, 3,391.

Smith, M.E. & Newman, H.W. (1959). J. biol. Chem. 234,1544.

Snitker, G. & Lintrup, J. (1967). Ugeskr. Laeg. 129,1587.

Solomon, D., Strummer, D. & Nair, P.P. (1967). Amer. J.

clin. Path. 48,295.

Song, C.S., Singer, J.W., Levere, R.D., Harris, D.S. &

Kappas, A. (1970). Clin. Res. 18,389.

Sonka, J., Gregorova, I., Pav, J. & Skrha, F. (1964).

Lancet, 2,44.

Sonka, J., Gregorova, I., Jiranek, M., Kölbél, F. & Matys, Z.

(1965). Endokrinologie, 47,152.

Southern, A.L., Gordon, G.G., Tochimoto, S., Kirkun, E.,

Krieger, D., Jacobson, M. & Kuntzman, R. (1969).

J. clin. Endocr. 29,251.

Stein, J.A. & Tschudy, D.P. (1970). Medicine, 49,1.

Stein, J.A., Tschudy, D.P., Corcoran, P.L. & Collins, A.
(1970). J. biol. Chem. 245,2213.

Stich, W. (1959). Schweiz. med. Wschr. 88,1012.

Stokvis, B.J. (1889). Ned. T. Geneesk. 25,409.

Strand, L.J., Felsher, B.F., Redeker, A.G. & Marver, H.S.
(1970). Proc. natn. Acad. Sci. 67,1315.

Strand, L.J., Manning, J. & Marver, H.S. (1971).

'Proceedings of the International Conference on Porphyrin
Metabolism and the Porphyrrias' (Special Issue, S. Afr. J.
Lab. clin. Med., 25th Sept., p.108.).

Taddeini, L. & Watson, C.J. (1968). Seminars Haematol.
5,335.

Taddeini, L., Nordstrom, K.L. & Watson, C.J. (1964).
Metabolism, 13,691.

Tait, J.F. (1963). J. clin. Endocr. 23,1285.

Talman, E.L., Labbe, R.F. & Aldrich, R.A. (1957). Arch. Biochem. Biophys. 66,289.

Tephly, T.R. & Mannering, G.J. (1968). Molec. Pharmacol. 4,10.

Thivolet, J., Perrot, H. & Arcadio, F. (1967). Dermatologica (Basel), 135,455.

Thomas, B.S. & Walton, D.R.M. (1968). J. Endocr. 41,208.

Thudichum, J.L. (1867). 10th Report of the M.D. of the Privy Council (London, H.M. Stationery Office, 1868).

Tio, T.H., Leijnsen, B., Jarret, A. & Rimington, C. (1957). Clin. Sci. 16,517.

Tomkins, G.M. (1956). Recent Progr. Hormone Res. 12,125.

Tomkins, G.M., Gelehrter, T.D., Granner, D., Martin, D., Samuel, H.H. & Thompson, E.B. (1969). Science, 166,1474.

Tschudy, D.P. (1968). Ann. N.Y. Acad. Sci. 151,850.

Tschudy, D.P. & Bonkowsky, H.L. (1972). Fedn. Proc. Amer. Soc. exp. Biol. 31,147.

Tschudy, D.P. & Collins, A. (1957). Science, 126,168.

Tschudy, D.P., Welland, F.H., Collins, A. & Hunter, G.W. (1964).
Metabolism, 13,396.

Tschudy, D.P., Perlroth, M.G., Marver, H.S., Collins, A., Hunter, G.
& Rechcigl, M. (1965). Proc. natn. Acad. Sci. 53,841.

Tschudy, D.P., Waxman, A. & Collins, A. (1967). Proc. natn.
Acad. Sci. 58,1944.

Tweedie, J.A. & Mattox, J.H. (1965). Obstet. Gynecol. 25,493.

Urquhart, (1898). J. ment. Sci. 44,313.

Vail, J.T. (1967). J. Amer. med. Ass. 201,671.

Vande Wiele, R.L., MacDonald, P.C., Gurpide, E. & Lieberman, S.
(1963). Recent Progr. Hormone Res. 19,275.

Vavra, J.D. (1967). J. clin. Invest. 46,1127.

Vestergaard, P. (1962). Acta endocrinol. Suppl. 64,3.

Vihko, R. (1966). Acta endocrinol. Suppl. 109,1.

Wada, O., Toyokawa, K. Urata, G., Yano, Y. & Nakao, K. (1969).
Biochem. Pharmacol. 18,1533.

Wajchenberg, B.L., Leme, C.E., Kieffer, J. & Pereira, V.G. (1969).

Clin. Sci. 36,15.

Waldenström, J. (1937). Acta med. scand. Suppl. 82.

Waldenström, J. (1939). Acta Psychiat. 14,375.

Waldenström, J. (1940). Svenska. Läkartidn. 37,1537.

Waldenström, J. (1957). Amer. J. Med. 22,758.

Waldenström, J. & Haeger-Aronsen, B. (1963). Brit. med. J.
2,272.

Walshe, M. (1963). Brit. J. Dermatol. 75,298.

Wang, D.Y. & Bulbrook, R.D. (1968). Adv. reprod. Physiol.
3,113.

Wang, D.Y., Bulbrook, R.D., Sneddon, A. & Hamilton, T. (1967).
J. Endocr. 38,307.

Wang, D.Y., Bulbrook, R.D., Thomas, B.S. & Friedman, M. (1968).
J. Endocr. 42,567.

Warin, R.F. (1963). Brit. J. Dermatol. 75,298.

Weissman, G. & Keiser, H. (1965). Biochem. Pharmacol. 14,537.

Weitzman, E.D., Fukushima, D., Nogueira, C., Roffwarg, H.,

Gallagher, T.F. & Hellman, L. (1971). J. clin. Endocr. 33,14.

Welland, F.H., Hellman, E.S., Gaddis, E.M., Collins, A.,

Hunter, G.W. & Tschudy, D.P. (1964). Metabolism, 13,232.

Wells, G.C. & Rimington, C. (1953). Brit. J. Dermatol. 65,337.

Wengle, B. (1964). Acta Soc. Med. "Deodecim", 69,105.

Westall, R.G. (1952). Nature, 170,614.

Wetterberg, L. (1964). Lancet, 2,1178.

Wetterberg, L., Geller, E. & Yuwiler, A. (1970). Biochem.

Pharmacol. 19,2833.

Whittaker, S.R.F. & Whitehead, T.P. (1956). Lancet, 1,547.

Wieland, R.G., Levy, R.P., Katz, D. & Hirschmann, H. (1963).

Biochim. biophys. Acta, 78,566.

Wieland, R.G., De Courcy, C., Levy, R.P., Zala, A.P. & Hirschmann, H.

(1965). J. clin. Invest. 44,159.

PUBLICATIONS

1. Hepatic δ -aminolaevulinic acid synthetase in an attack of hereditary coproporphyria and during remission.
McIntyre, H., Pearson, A.J.G., Allan, D.J., Craske, S., West, G.M.L., Moore, H.R., Beattie, A.D., Paxton, J.W. & Goldberg, A.
The Lancet (1971) 1, 560
2. 17-oxosteroid control of porphyrin biosynthesis.
Moore, H.R., Paxton, J.W., Beattie, A.D. & Goldberg, A.
Enzyme - in press.
3. Abnormalities of 17-oxosteroid conjugate amounts in human hepatic porphyria.
Paxton, J.W., Moore, H.R., Beattie, A.D. & Goldberg, A.
Biochemical Society Transactions - in Press
4. 17-oxosteroids in acute intermittent porphyria.
Paxton, J.W., Moore, H.R., Beattie, A.D. & Goldberg, A.
Clinical Science - submitted.
5. 17-oxosteroids and porphyrins in hepatic porphyria.
Paxton, J.W., Moore, H.R. & Goldberg, A.
In Preparation.